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Universidad del País Vasco | Euskal Herriko Unibertsitatea

FACULTY OF PHARMACY
UNIVERSITY OF THE BASQUE COUNTRY

Conventional microbiological diagnosis and epidemiology of bacteraemia in a tertiary and a district hospital over a six-year period

Diagnóstico microbiológico convencional y epidemiología de la bacteriemia en un hospital terciario y en uno comarcal durante un periodo de 6 años

Tesis doctoral

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Vitoria-Gasteiz, 2022

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Versión del Protocolo: Versión 2. 27 de julio 2021
Versión de la HIP: GENERAL / exención

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AGRADECIMIENTOS

Este trabajo no hubiera sido posible sin la ayuda, colaboración y apoyo de mi familia, compañeros y amigos.

A Andrés Canut, por proponerme y animarme al desarrollo de esta tesis. Su optimismo ha sido clave en el desarrollo de la misma.

A Javier Garaizar por su cercanía, colaboración y apoyo brindados desde el primer momento.

A todos mis compañeros del Servicio de Microbiología del HUA por apoyarme y por estar siempre dispuestos a echarme una mano.

A Ismael Barbero por compartir sus conocimientos y por su colaboración.

A mi familia, y en especial a mi madre, por apoyarme incondicionalmente en todos los proyectos en los que me embarco.

Y a Alberto, por su apoyo moral y por darme impulso cuando lo necesitaba.

Muchas gracias a todos.

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ABBREVIATIONS

Abbreviation	*Full name*
AFG	Anidulafungin
AMB	Amphotericin B
AMC	Amoxicillin-clavulanate
AMI	Amikacin
AMP	Ampicillin
ASP	Antimicrobial stewardship program
AST	Antimicrobial susceptibility testing
AZT	Azithromycin
BC	Blood culture
BCC	Blood culture contamination
BEN	Benzylpenicillin
BFG	*Bacteroides fragilis* Group
BLR	Binary logistic regression
BSI	Bloodstream infection
CA	Categorical agreement
CEP	Cefepime
CFU	Colony forming unit
CI	Confidence interval
CIP	Ciprofloxacin
CIX	Cefixime
CLI	Clindamycin
CLSI	Clinical and Laboratory Standards Institute
CO	Community-onset
CoNS	Coagulase-negative staphylococci
COVID-19	Coronavirus disease 2019

CTA	Cefotaxime
CTZ	Ceftazidime
CUR	Cefuroxime
DAP	Daptomycin
DGI	Disseminated gonococcal infection
ECCMID	European Congress of Clinical Microbiology and Infectious Diseases
ECOFF	Epidemiological cut-off value
ED	Emergency Department
ERT	Ertapenem
ESBL	Extended-spectrum beta-lactamase
ESKAPE	*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FLC	Fluconazole
GBS	Group B *Streptococcus*
GEN	Gentamicin
GI	Gastrointestinal
HACEK	*Haemophilus* spp., *Aggregatibacter* spp., *Cardiobacterium* spp., *Eikenella* spp. and *Kingella* spp.
HAD	Hospital of Alto Deba
HCA	Healthcare-associated
HIV	Human immunodeficiency virus
HO	Hospital-onset
HUA	University Hospital of Araba
ICU	Intensive care unit
ICT	Immunochromatographic
IMI	Imipenem
IQR	Interquartile range

ITC	Itraconazole
IV	Intravenous
LEV	Levofloxacin
LIN	Linezolid
M	Male
MA	Mean age
MALDI TOF MS	Matrix-assisted laser desorption ionization time of flight mass spectrometry
MAX	Maximum
MDR	Multi-drug resistance
mE	Minor error
ME	Major error
MER	Meropenem
MET	Metronidazole
MFG	Micafungin
MIC	Minimum inhibitory concentration
MIN	Minimum
MLST	Multilocus sequence typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-susceptible *Staphylococcus aureus*
n	Number
NFGNB	Non-fermenting Gram-negative bacilli
NR	Not readable
NS	Not significant
OXA	Oxacillin
PBDP	Positive bottle detection pattern
PCR	Polymerase chain reaction
PIT	Piperacillin-tazobactam
POS	Posaconazole
RAST	Rapid antimicrobial susceptibility testing
RT-PCR	Reverse transcription PCR

SARS-CoV-2	Severe acute respiratory syndrome coronavirus-2
SD	Standard deviation
TEI	Teicoplanin
TET	Tetracycline
TOB	Tobramycin
TRS	Trimethoprim-sulfamethoxazole
TTP	Time-to-positivity
VAN	Vancomycin
VME	Very major error
VRC	Voriconazole
WGS	Whole genome sequencing

RESUMEN

RESUMEN DE LA TESIS DOCTORAL:

Diagnóstico microbiológico convencional y epidemiología de la bacteriemia en un hospital terciario y en uno comarcal durante un periodo de 6 años

La sepsis es un síndrome clínico de extrema gravedad, con una tasa de mortalidad elevada, una incidencia creciente e importantes costes asociados. En todo el mundo, la sepsis sigue siendo una de las causas más importantes de morbimortalidad.

Las tasas de morbilidad y mortalidad de las infecciones del torrente sanguíneo están relacionadas con el retraso en la administración del primer antimicrobiano apropiado, y varios estudios han demostrado que la administración temprana de una terapia empírica adecuada es crucial para la supervivencia de los pacientes con sepsis.

Se estima que el alrededor de la mitad de los pacientes adultos con bacteriemia (y el 70% de los pacientes con fungemia) reciben un tratamiento antimicrobiano empírico incorrecto. Estos tratamientos se eligen en función de los datos clínicos y epidemiológicos locales, por lo que las guías de tratamiento empírico deben adaptarse, revisarse y actualizarse periódicamente.

Dado que el tratamiento empírico se basa en la epidemiología local, es fundamental el seguimiento continuo de las tendencias temporales en la tasa de incidencia, la distribución de especies y los perfiles de sensibilidad a antimicrobianos clave. El

tratamiento antibiótico se inicia con frecuencia en los Servicios de Urgencias y tiene una gran trascendencia para el paciente y su evolución. Por ello, conocer la epidemiología y la etiología de los aislamientos obtenidos en hemocultivos solicitados en estos servicios es especialmente importante.

Además de enfatizar el valor de la terapia empírica temprana, es importante garantizar que las infecciones del torrente sanguíneo se diagnostican correctamente y que los microorganismos causales, sus sensibilidades y las posibles fuentes primarias de infección se evalúan minuciosamente para permitir una terapia antimicrobiana dirigida óptima. Por lo tanto, el diagnóstico microbiológico rápido y preciso del agente causal y su perfil de sensibilidad es crucial.

Para el diagnóstico de la infección del torrente sanguíneo los hemocultivos continúan siendo la herramienta de primera línea y es previsible que continúen siéndolo en un futuro próximo a pesar de los avances en nuevas tecnologías. La identificación rápida y las pruebas de sensibilidad antimicrobiana de los hemocultivos positivos se encuentran entre las tareas más importantes que el Laboratorio de Microbiología Clínica lleva a cabo y se están realizando grandes esfuerzos para acelerar el diagnóstico microbiológico de las bacteriemias. Sin embargo, cabe enfatizar que, aunque la automatización o la utilización de tecnologías tales como la espectrometría de masas (MALDI-TOF) o las plataformas moleculares recientemente comercializadas han permitido ya una aceleración dramática en el diagnóstico microbiológico, sin un Laboratorio de Microbiología operativo las 24 horas del día, los 7 días de la semana y un microbiólogo de guardia que emita informes, estas mejoras pueden ser ineficaces.

Por otra parte, el valor pronóstico de los hemocultivos está limitado por la contaminación, definida como el crecimiento en cultivo de microorganismos que no están realmente en la sangre del paciente. Las contaminaciones representan hasta el 50% de los hemocultivos positivos y pueden conducir a tratamientos antibióticos innecesarios, pruebas de laboratorio redundantes, hospitalizaciones evitables, aumentos en la estancia hospitalaria y cambios innecesarios de vías, entre otros. Por ello, el desarrollo de herramientas que puedan ayudar a dilucidar el papel que juega un microorganismo aislado en la infección es especialmente importante en instituciones con una alta tasa de contaminaciones.

El tiempo de positivización de hemocultivos constituye una información fácilmente accesible proporcionada por los sistemas automatizados actualmente disponibles, que se ha utilizado durante mucho tiempo como herramienta diagnóstica en la bacteriemia relacionada con el catéter. Además, el tiempo de positivización puede reflejar el tipo y la especie de microorganismo aislado y la concentración o carga bacteriana del microorganismo en la sangre. De ahí que su uso potencial en la práctica clínica diaria haya sido cada vez más reconocido.

Este trabajo se centra en el estudio del diagnóstico microbiológico de bacteriemia y fungemia por cultivo, analizando la epidemiología, etiología y perfiles de resistencia de los principales microorganismos responsables de infección del torrente sanguíneo a antibióticos clave aislados en el Laboratorio centralizado de Microbiología del Hospital Universitario de Araba durante un período de seis años (2015-2020), evaluando dos ensayos rápidos de antibiograma directo, e investigando el papel potencial del tiempo de positivización en el diagnóstico de bacteriemia. Así mismo, se evalúa el impacto de la

pandemia por SARS-CoV-2 en la utilización de hemocultivos y se describen las características de la bacteriemia en pacientes infectados con SARS-CoV-2.

El Laboratorio brinda servicio a los pacientes que reciben atención en una red de hospitales que comprende el Hospital Universitario de Araba, que incluye las sedes de los hospitales Txagorritxu y Santiago Apóstol (HUA), y los hospitales comarcales Alto Deba en Mondragón (Gipuzkoa) y Leza en la Rioja Alavesa. En este trabajo, dado el reducido número de muestras pertenecientes a este último hospital, las muestras procedentes del mismo se han incluido dentro del HUA.

El Hospital Universitario de Araba es un hospital terciario que atiende a una población de aproximadamente 340000 habitantes, los ingresos hospitalarios anuales promedio fueron 40152 durante el período de estudio y tiene 734 camas estándar y 30 camas de cuidados intensivos. Por otro lado, el Hospital de Alto Deba es un hospital comarcal, da cobertura a unos 65000 habitantes, la media anual de ingresos hospitalarios fue de 5471 durante el periodo de estudio y cuenta con 74 camas.

Los objetivos de esta tesis doctoral han sido:

1. Evaluar las tendencias en la carga de trabajo, la incidencia, la etiología y los perfiles de resistencia a antibióticos clave en bacteriemia durante un período de seis años (2015-2020) tanto en un hospital terciario (Hospital Universitario de Araba), como en uno comarcal (Hospital de Alto Deba). Evaluar los episodios de bacteriemia pertenecientes a hemocultivos solicitados en los Servicios de Urgencias.

2. Evaluar dos métodos de antibiograma rápido utilizando E-tests y VITEK®-2 para una detección rápida y una diferenciación precisa de la sensibilidad antimicrobiana en enterobacterias.
3. Analizar el tiempo de positivización de los hemocultivos durante un período de dos años (2019-2020): i) evaluar el tiempo de positivización de todos los episodios de bacteriemia (verdaderos y contaminaciones) aislados durante el periodo de estudio para examinar en qué medida el tiempo de positivización puede aportar información sobre el tipo de microorganismo aislado, el foco y su implicación en la infección; ii) analizar aquellos episodios en los que el tiempo de positivización fue superior a 24 h; iii) analizar por separado todos los episodios de candidemia y determinar la relación entre el tiempo de positivización y la mortalidad a los 28 días, y iv) explorar la influencia del tiempo de positivización y el patrón de positivización de los frascos en episodios contaminados por estafilococos coagulasa-negativos utilizando análisis de regresión logística binaria y diseñar una fórmula de predicción.
4. Evaluar el impacto de la pandemia por SARS-CoV-2 en la utilización de hemocultivos y describir las características de la bacteriemia en pacientes infectados con SARS-CoV-2.

Para el primer objetivo, de 2015 a 2020 se investigaron un total de 96157 hemocultivos y se identificaron un total 5426 episodios de bacteriemia y fungemia. El número de hemocultivos solicitado por 1000 ingresos se mantuvo estable en ambos hospitales. En el Hospital Universitario de Araba, la incidencia de infecciones del torrente sanguíneo por 1000 ingresos evolucionó de 18.0 episodios en 2016 a 22.7 en 2020, lo que muestra un incremento anual de 0.77 episodios por 1000 ingresos (intervalo de confianza (IC) del

95%, 0.12-1.42; *P* = 0.031). Los episodios pertenecientes a hemocultivos solicitados en los Servicio de Urgencias aumentaron significativamente en el hospital terciario, con un incremento anual de 0.56 episodios por 1000 ingresos (IC del 95%, 0.18-0.93; *P*=0.015). Estas tendencias no se observaron en el hospital comarcal. La incidencia de episodios causados por enterobacterias productoras de beta-lactamasas de espectro extendido y por *Pseudomonas aeruginosa* multirresiste no aumentó a lo largo de los años, con tasas que variaron de 0.7 a 1.5 episodios por 1000 ingresos y de 0.02 a 0.2 episodios por 1000 ingresos, respectivamente. La tasa de *Staphylococccus aureus* resistente a la meticilina se mantuvo estable. La resistencia a glicopéptidos y linezolid siguió siendo un evento raro entre las cepas de *Enterococcus faecalis* y *E. faecium*.

Para el segundo objetivo, de marzo a diciembre de 2019 se evaluaron prospectivamente un total de 121 muestras de hemocultivos positivos en las que se aislaron enterobacterias.

Mediante la técnica rápida de E-test evaluada, transcurridas 5 horas de incubación, el 95.0% tenía concentraciones mínimas inhibitorias legibles y, después de 7 horas, el 99.2%. En concreto para *E. coli*, la concentración mínima inhibitoria fue legible a las cinco horas en todos los casos excepto en uno. Para el ensayo VITEK®-2 analizado, la mayoría de las concentraciones mínimas inhibitorias estuvieron disponibles después de 7 horas, excepto para imipenem y piperacilina-tazobactam. Para ambos métodos evaluados, el acuerdo categórico más bajo se obtuvo para amoxicilina-clavulánico. Los datos obtenidos mostraron un 0.8% de errores mayores para el método basado en E-test. En el ensayo VITEK®-2, la tasa de errores mayores más alta se obtuvo para la gentamicina y fue del 3.3%.

Para el tercer objetivo, entre enero de 2019 y diciembre de 2020, se registraron prospectivamente los tiempos de positivización del primer frasco positivo de todos los episodios de bacteriemia y fungemia reales y contaminados.

La mediana de tiempo de positivización para todos los episodios reales fue de 12.1 (rango intercuartílico [IQR], 10.1-16.8) horas. El 49.4% creció en menos de 12 horas, y el 87.3% lo hizo en menos de 24 horas. El 96.2% de los frascos pertenecientes a episodios reales se positivizaron en menos de 48 horas. Los frascos de hemocultivo pertenecientes a episodios verdaderos se positivizaron significativamente más rápido (P<0.0001) que los considerados contaminaciones. La probabilidad de una verdadera infección del torrente sanguíneo fue del 98.1% (IC 95%, 96.9-98.9%) cuando el tiempo de positivización fue inferior a 12 horas, y del 71.3% (IC 95%, 69.4-73.3%) cuando el tiempo de positivización fue menor a 24 horas. Cuando un frasco se positivizó en el rango de 24-48 h, la probabilidad de contaminación fue del 69.7% (IC 95%, 65.8-73.8%). Se propuso una fórmula de regresión logística binaria para predecir la probabilidad de contaminación dependiendo del tiempo de positivización y del patrón de positivización y se ajustó en una hoja Excel lo que permite al usuario introducir diferentes variables.

Sobre el cuarto objetivo, el 28 de febrero de 2020 se diagnosticó microbiológicamente en nuestro laboratorio el primer caso de SARS-CoV-2. La rápida propagación de este virus en nuestra área sanitaria provocó una oleada de pacientes febriles que acudieron a los hospitales de la red.

El número de hemocultivos solicitados por 1000 ingresos en 2020 aumentó estadísticamente (P=0.018) en 5.1% (IC 95%,1.5-9.9; P=0.011) en comparación con 2019, con un pico marcado registrado en marzo.

Excluyendo los episodios dudosos o no concluyentes el número de episodios reales recuperados fue similar en ambos años. El número de hemocultivos contaminados aumentó en 2020, aunque el incremento no fue significativo. En pacientes con SARS-CoV-2, tanto el espectro de microorganismos implicados como el foco de infección fueron los esperados para pacientes con hospitalizaciones prolongadas.

INTRODUCTION

Bloodstream infections (BSI) are of increasing public health concern with an estimated burden of over 1,2 million episodes of BSI and 157000 deaths per year in Europe (1). Worldwide, sepsis remains one of the most important causes of both morbidity and mortality with an estimated nineteen million cases and up to five million deaths annually (2).

The morbidity and mortality rates of BSI are related with the delay in administration of the first appropriate antimicrobial agent, several studies having demonstrated that early administration of adequate empirical antimicrobial therapy is crucial for the survival of patients with sepsis (3–6). Hence, among patients with BSI, receiving inappropriate empirical antibiotic therapy is associated with increased mortality (7).

Empirical antimicrobial treatments and regimens are chosen based on clinical and local epidemiological data and antibiotic guidelines should be adapted, reviewed, and updated periodically. Within Intensive care units (ICUs) antimicrobial use is generally empirical since the source of infection in seriously ill patients is not always easily defined. In such situations the physician in charge faces a dilemma: whether to prescribe broad-spectrum empirical antimicrobials with the risk of contributing to further resistance selection, or to use narrow-spectrum empiric treatments and assume the risk of not covering the microorganism involved (8). Either way, whenever possible, empirical treatment will be initiated after blood culture (BC) sampling.

Since empirical therapy relies on local epidemiology, continuous regional monitoring of temporal trends in incidence rates, species distribution, and susceptibility profiles to key

antimicrobials is crucial. Antimicrobial treatment is frequently initiated in the Emergency Department (ED) and has therefore a significant impact on the patient and their evolution (9).

In addition to emphasizing the value of early empirical therapy, it is important to ensure that BSIs are diagnosed accurately and that infecting pathogens, their antimicrobial susceptibilities, and the possible primary sources of infection are evaluated thoroughly, to enable optimal targeted therapy. Therefore, rapid and accurate microbiological diagnosis of the causative microorganism and its susceptibility profile is extremely important.

For the diagnosis of BSI BCs remain the first-line tool and are expected to remain so for the foreseeable future despite the advancement in new technologies. Rapid identification and antimicrobial susceptibility testing (AST) of positive BCs is among the most important tasks of the clinical microbiology laboratory and great efforts are being made to speed up the microbiological diagnosis of BSIs. Nevertheless, the prognostic value of BCs is limited by contamination, defined as the growth in culture of microorganisms that are not actually in the blood. Contaminations represent up to 50% of positive BCs and can lead to unnecessary antibiotic treatments, redundant laboratory testing, avoidable hospitalizations, increase in the patient's length of stay and unnecessary removal of lines. Hence, the development of tools that can help elucidate the role that an isolated microorganism plays in infection is especially important in institutions with a high contamination rate.

BC time-to-positivity (TTP) is an easily accessible information provided by currently available automated BC systems that has long be used as a diagnostic tool for catheter-

related bacteraemia. The TTP can reflect the type and species of microorganism isolated and the concentration or bacterial load of the microorganism in blood. In addition, it can often suggest the type of role the microorganism plays in the infection, as it can help determine whether the isolated microorganism is likely a contamination. Thus, its potential use in the daily clinical practice has been increasingly acknowledged.

Brief history of blood cultures

It remains unknown when exactly BCs started to be performed. Prior to 1880 BC was limited to one drop of blood obtained from the fingertip and it was mainly used in puerperal fever and endocarditis (10).

As early as 1880 it was recognized that the quantity of bacteria in blood was low and that collecting a sufficient volume was critical, suggesting the number of pricks should be increased. When the sterilisable syringe was invented (ca 1886), physicians were able to aseptically draw larger volumes of blood and the BC technique progressed (10).

Early 20th century, the process of blood culturing was a widespread practice among septic patients, and detailed recommendations for patient's skin preparation, blood volume to be withdrawn, media and number of flasks to be inoculated were published. Until the 1970s, no major changes above the classical BC technique were made (10).

Based on the principle of radiospirometric detection of bacteria developed by the National Aeronautics and Space Administration (NASA) to identify microbial life in Martian soil, the first automated BC technology, the Bactec™ growth detector (Becton Dickinson) was invented. Based on the colorimetric detection of pH changes secondary to microbial growth and introduced in 1990, the BacT/Alert (Organon Teknika Corporation) was the first continuously monitored, fully automated BC system (11). The ESP BC system (Difco) (12) launched in 1994, monitored changes in the pressure of the bottle headspace secondary to microbial metabolism via an electronic pressure sensor connected to the septum of the bottle through a sterile vent, and the VITAL system introduced by bioMérieux, incorporated a fluorescent molecule the conformation of

which altered because of pH changes, redox potential variations, and CO_2 production (13). Thus, the beginning of the era of continuous BC monitoring was marked together by BacT/Alert, BACTEC 9000 series, ESP and VITAL.

Definitions and classification

BSI is generally considered to be present when an organism associated with disease is cultured from the blood of an infected patient. Thus, BSIs are those produced by the presence of viable microorganisms in the blood as evidenced by growth in BC where contamination has been ruled out. The presence of viable bacteria in blood is defined as bacteraemia (the suffix "–emia" refers to the blood), whereas the term fungaemia refers to the presence of fungi in the blood.

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection, while septic shock is defined as a subset of sepsis in which particularly profound circulatory, cellular and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone (14). The World Health Organization has urged member states to strengthen efforts to identify, document, prevent, and treat sepsis.

Onwards and to simplify the reading, the term bacteraemia will include fungaemia, unless otherwise indicated. Bacteraemia can be classified according to the place of acquisition, the origin, the number of microorganisms involved, and the clinical pattern (Table 1).

Place of acquisition

Depending on the site of acquisition and risk factors, BSIs can be classified as community-onset (CO), hospital-onset (HO) or nosocomial, and healthcare-associated (HCA) BSI.

CO bacteraemia is defined as clinically relevant positive BCs taken within 48 hours of hospitalization, without a hospital stay in the 30 days prior to admission nor any healthcare assistance activity, or after 48 hours if the clinical presentation and identified

pathogens are consistent with CO disease. Several authors have estimated that about 40% of the BSI episodes are CO (15,16), although the figure may be lower depending on the classification criteria used (17).

HO bacteraemia or nosocomial bacteraemia (*nosos*: disease, *komein*: to take care of, *nosokomein*: hospital) refers to clinically significant positive BCs drawn after more than 48 hours of hospitalization and if no evidence of infection was present on admission (18).

HCA bacteraemia is defined as BSI that is diagnosed in the first 48 hours of hospitalization in patients with at least one of several recent exposures, including intravenous (IV) therapy or other nursing care at home services, outpatients with indwelling urinary or IV catheters, patients on hemodialysis or peritoneal dialysis in the previous 30 days, hospitalization in an acute care hospital in the preceding 90 days, or residence in a nursing home or any long-term care facilities (17,18).

Source of infection

Depending on the origin, bacteraemia can be classified as primary or of unknown origin, and secondary to an infection that is generally microbiologically documented.

The most common source of CO BSIs is the urinary, lower respiratory, and gastrointestinal (GI) tract, whereas HCA and HO BSIs are more often catheter-related infections. Nevertheless, even with an exhaustive diagnostic procedure, the origin remains unknown in about 11-23% (19–22) of patients.

Number of microorganisms involved

Depending on the number of microorganisms involved, BSIs can be classified as mono- or polymicrobial, when the bacteraemia episode is due one or to at least two different organisms isolated from the same blood set or within 48 h of each other, respectively. The incidence of polymicrobial BSI in the general population ranges from 6%-14%, but increases in patients with cancer, reaching up to 32% (23–26).

Time of appearance

Depending on the clinical pattern, BSIs can be divided in transient, intermittent, and persistent.

Transient bacteraemia lasts for minutes or a few hours and is the most frequent one; it appears at the beginning of certain local bacterial infections such as pneumonia, meningitis, and complicated urinary tract infections or during procedures in contact with contaminated mucous membranes (during dental procedures, after GI biopsy, after percutaneous catheterization of the vascular system, bladder, or common bile duct, after surgical debridement or drainage).

Intermittent bacteraemia develops when periodical recurrences occur by the same microorganism, or, in other words, is a bacteraemia episode due to the same microorganism that is detected intermittently in the same patient because of a cycle of clearance and recurrence.

Persistent bacteraemia is the one that comes along with endocarditis or other endovascular infections such as suppurative thrombophlebitis or other infections originated from an infected intravascular catheter. Persistent bacteraemia also occurs

during the early stages of systemic bacterial infections, such as brucellosis and typhoid fever.

Finally, "gap bacteraemia" is the one that takes place even though the patient is receiving an adequate antibiotic treatment after negative control BC (27).

Table 1. Definitions of BSI and associated entities.

ENTITY	DEFINITION
BSI	Bacteraemia/fungaemia that is associated with infection.
Bacteraemia	Presence of viable bacteria in the blood.
Fungaemia	Presence of viable fungi in the blood.
BC contamination	Growth of bacteria in BCs that are not present in the patient's bloodstream.
Sepsis	Life-threatening organ dysfunction caused by a dysregulated host response to infection.
Septic shock	A subset of sepsis in which particularly profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone.
CO BSI	BSI occurring in an outpatient or first identified (culture drawn) < 48 h following admission to hospital.
HO BSI	BSI that is first identified (culture drawn) $\geq$ 48 h after hospital admission and within 48 h following hospital discharge.
HCA BSI	CO BSI associated with significant prior health care exposure (as evidenced by recent hospitalization, specialized in-home medical services, care in hospital-based clinic or haemodialysis unit, or residence in a nursing home).
Polymicrobial BSI	BSI episode that is due to at least two different organisms isolated from the same blood set or within 48 h of each other.
Transient bacteraemia	Bacteraemia that appears at the beginning of certain local bacterial infections such as pneumonia, meningitis, and complicated urinary tract infections or during procedures in contact with contaminated mucous membranes.

Intermittent bacteraemia	Bacteraemia episode due to the same microorganism that is detected intermittently in the same patient because of a cycle of clearance and recurrence.
Persistent bacteraemia	Bacteraemia that comes along with endocarditis or other endovascular infections such as suppurative thrombophlebitis or other infections originated from an infected intravascular catheter.
Gap bacteraemia	Bacteraemia episode that takes place even though the patient is receiving adequate antibiotic treatment after negative control BCs.

BSI: bloodstream infection, BC: blood culture, CO: community-onset, HO: hospital-onset, HCA: healthcare-associated.

Indications for performing blood cultures

There is no universal recommendation on the indication for taking BCs. In general terms, BCs should be obtained whenever a suspicion of bacteraemia exists, including hospitalized patients and selected outpatients with fever (body temperature ≥ 38 °C) and leucocytosis, leukopenia or thrombopenia not related to haematological processes. Nevertheless, bacteraemia may be present in the setting of normothermia and/or a normal white blood cell count. Circumstances in which BCs are especially important include sepsis, meningitis, endocarditis, pneumonia, arthritis, osteomyelitis, peritonitis, and fever of unknown origin (hidden abscess, typhoid fever, brucellosis, tularaemia ...). In addition, BCs should always be drawn whenever a catheter is removed and sent to be cultured on suspicion of catheter-related bacteraemia. On the other hand, the extraction of BCs is also indicated in young children or the elderly who experience a sudden decline, since the typical signs and symptoms of bacteraemia may not be present in these populations.

Indications for BCs:

- suspicion of bacteraemia or fungaemia.
- Signs and symptoms of sepsis: tachycardia, tachypnoea, fever or hypothermia, hypotension, prostration.
- Leucocytosis.
- Granulocytopenia.
- Endocarditis.
- Fever of unknown origin.

- Systemic and localized infections: meningitis, acute bacterial pneumonia, septic arthritis, osteomyelitis, septic arthritis …
- Young children or the elderly who experience a sudden decline.

Laboratory diagnosis of BSI

The quality of the specimens submitted to the microbiology laboratory is critical for optimal specimen evaluation. Laboratory diagnosis of bacteraemia depends primarily on BCs, which are one of the most important cultures carried out by the microbiology laboratory. The goal of the microbiology laboratory is to provide accurate, clinically pertinent results in in the shortest possible time.

Only if the specimen obtained is appropriate for processing, valid interpretation of the results of culture can be achieved. Therefore, major efforts should be made to collect only those specimens that may yield pathogens, rather than colonizing flora or contaminants. Quality BC results, and, in general, of any culture that is processed in the microbiology laboratory relies upon quality throughout the three phases of laboratory testing: preanalytical, analytical and postanalytical phases. Most errors are known to occur in the preanalytical phase (prior to laboratory analysis). In this stage, the laboratory has no direct control on the process and only if the preanalytical factors are carefully controlled, optimal detection of microorganisms is achieved.

There are different conceptions of what a BC constitutes. The definition used in this document dictates as follows: a BC is a volume of blood from a single phlebotomy obtained under aseptic conditions that is inoculated into one or more bottles or vials containing broth culture medium. Thus, one BC usually consists of blood from a single venepuncture inoculated into two separate bottles to accommodate the volume of the blood removed.

Factors that can influence the recovery of microorganisms from the blood include the timing of blood collection, the number of sets collected, and the volume of blood, where the latter is believed to be the major factor influencing successful recovery of pathogens. It is essential that phlebotomists or nurses in charge of BC extraction are properly educated about the time and anatomical sites of extraction, optimal volume of blood to be obtained, the atmosphere of the culture bottles, number of extractions and aseptic conditions that must be followed. Furthermore, to reduce variation in the collection of BC samples it is recommended to follow the instructions of each manufacturer for filling the bottles.

Timing of blood collection

In case of acute infection, the time of blood collection should match that in which there is a greater number of viable bacteria in the blood, which is known to be the time that precedes the onset of fever. Therefore, it is recommended that the extraction coincides with the appearance of chills or that this occurs within two hours of the appearance of the same (28), since it is believed that chills usually precede immediately to the feverish peak and appears approximately one hour after the microorganism enters the bloodstream (29).

BC should be drawn, whenever possible, prior to initiation of antimicrobial therapy. If that is not possible, extraction should be performed when the antibiotic is in its trough concentration, this is just before of the next dose.

Sites of blood extraction

Blood should be drawn by venepuncture (peripheral extraction) from veins in the antecubital fossae, avoiding extraction from intravascular devices, changing from equipment and anatomical site in the extraction of each BC. Only when line-related infections are suspected, blood should be taken from a central line, Hickman line, arterial line or other lines. These specimens should all be collected at the same time as a peripheral BC and carefully labelled with the site and time of collection (30).

Number of sets

In general, patients with bacteraemia and even in the event of sepsis, are likely to have low quantities of bacteria in the blood. It is estimated that during an episode of bacteraemia the number of microorganisms present in the blood ranges from 10 colony forming units (CFU)/mL to 10^4 CFU /mL and may even be less than 0.1 CFU/mL in 20% of cases (31,32). Besides, as bacteraemia in adults is generally intermittent, multiple BCs, each containing large volumes of blood, are required to properly detect bacteraemia. This is not the case of endocarditis or septic thrombophlebitis, where all the bottles are typically positive. In general, it is recommended to obtain at least two sets each containing one aerobic and one anaerobic BC bottle, from different peripheral sites. Published data show that a single BC does not provide sufficient yield and, so, that improved microbial yield of clinically significant microorganisms is achieved by performing paired BC instead of single BCs (33); besides, interpretation of a single BC may be cumbersome and even impossible when microorganisms that can both be true pathogens or contaminants are isolated (e.g., coagulase-negative staphylococci [CoNS]). Therefore, the practice of drawing a single BC ("single sample strategy") should be strongly discouraged.

In patients in whom blood collection is difficult, it is still recommended to first fill an aerobic flask with the available blood volume and only then inoculate any residual blood volume into an anaerobic flask. Similarly, in paediatric patients it is recommended to inoculate the entire blood specimen into an aerobic BC bottle.

Type of BC bottle

Although the routine use of anaerobic BC media was discouraged for a time, more recent data indicate that anaerobic bacteraemia is common enough to warrant routine use of these media. Thus, in routine clinical practice the use of a combination of aerobic and anaerobic BC media is recommended. The low prevalence of paediatric anaerobic bacteraemia supports the practice of paediatric anaerobic BC only for those at increased risk, such as immunocompromised patients and those with head, neck, and intraabdominal infections (34,35).

Most *Candida* species grow in conventional aerobic BC, but when there is a high suspicion of fungaemia, the use of specialized media has shown to increase the recovery rate and to reduce the detection time (36).

Volume of blood

Sufficient volume of blood is a critical factor for the successful recovery of organisms causing BSI and determines the sensitivity, specificity, and TTP of a BC. Several studies have shown that volume is the most crucial factor in increasing microbial recovery rates from blood. But, in any case, the extraction of a large volume of blood should not pose a risk to the patient. Therefore, there must always be a balance between the need for an

accurate microbiological diagnosis and the risk for patients of acquiring nosocomial anemia (37).

Historically, it has been believed that bacteraemia in the paediatric population was associated with a high bacterial load. However, the incidence of low-level bacteraemia in these patients is presumed to be more common than has been believed and is estimated to occur in 38% to 68% of all paediatric patients with a positive BC (38,39).

In general, for adults the target volume is standardized in 8-10 mL per bottle (40). When it comes to paediatric populations this volume might not be available, especially in younger age groups. It is generally accepted that blood collection in children should be based on the age and/or weight of the patient, both of which are interdependent variables and there are several recommendations based on these parameters. Nevertheless, there is a wide range of different recommendations for optimal blood volume for the various age and weight groups (Table 2) (41).

Table 2. Recommendations for optimal blood volume in paediatric populations based on weight and age classes. *Adapted from Huber et al.* (41)

PATIENT WEIGHT (kg)	TOTAL BLOOD VOLUME (mL)
≤ 2.0	1.0–4.5
> 2.0–5.0	1.0–6.0
> 5.0–10.0	1.5–10.0
> 10.0–20.0	6.0–23.0
> 20.0–30.0	≥ 10.0
PATIENT AGE (YEARS)	**TOTAL BLOOD VOLUME (mL)**
< 1	> 0.5–3.0
≥ 1–3	1.0–4.0
> 3–10	3.0– 8.0
≥ 10	20.0

Processing BCs

During the past forty years there have been numerous changes in BC media and incubation systems. Newer media are more sensitive for the detection of microorganisms, and modern automated BC systems detect positive results earlier than previously used conventional BC systems (42). Current automated BC incubation systems incubate blood specimens inoculated into BC bottles and signal when growth is detected.

The main automated BC systems currently commercially available and their positivity detection system are presented in Table 3.

Table 3. Main automated BC incubation systems currently commercially available.

SYSTEM	POSITIVITY DETECTION SYSTEM
BD BACTEC (BD Diagnostics)	Fluorescent sensor of CO_2 production
BacT/ALERT 3D (bioMérieux)	Colorimetric sensor of CO_2 production
VersaTREK (Trek Diagnostic Systems, ThermoFisher)	Monitoring of redox variations

For each of these systems, different formulations of BC media are available. In addition to containing a rich media for the recovery of aerobic and anaerobic bacteria, respectively, BC bottles often contain resins or charcoal particles intended to neutralize antibiotics or other inhibitory substances that may be present in the patient's blood that could potentially hinder the bacterial growth; BACTEC uses proprietary binding resin beads, while BacT/Alert utilizes Ecosorb, a blend of Fuller's earth and activated charcoal (43). Blood-to-broth ratios range between 1:2.5 and 1:6, depending on whether agents that bind or sequester antimicrobials are present in the bottles.

To reduce or eliminate clotting, BC bottles require the use of an anticoagulant. Sodium polyanethole sulphonate (SPS) is the anticoagulant contained in most of the BC bottles currently marketed. Others contain sodium citrate, alone or in combination with SPS. SPS, in addition to acting as an anticoagulant, improves the rate and speed of bacterial isolation, inhibits the activity of complement and lysozyme present in the blood, inhibits phagocytosis, and inactivates some aminoglycoside agents. But, in addition, it can reduce microbial recovery rates; in fact, it may inhibit, totally or partially, the growth of *Neisseria meningitidis*, *Gardnerella vaginalis*, *N. gonorrhoeae*, *Peptostreptococcus anaerobius* and *Streptobacillus moniliformis* (44–46).

Standard incubation period is of five days. However, recent studies reveal that incubation periods of four days could be sufficient to recover microorganisms really involved in infection, even those belonging to the HACEK group (i.e., *Haemophilus* spp., *Aggregatibacter* spp., *Cardiobacterium* spp., *Eikenella* spp. and *Kingella* spp.), and to minimize contaminations (47,48). In fact, published data do not support the widespread practice of prolonging incubation times for cases of suspected endocarditis due to fastidious microorganisms. Extended incubation remains useful to detect *Cutibacterium acnes* in individuals with prosthetic-valve endocarditis or other clinical conditions in which it may actually be involved, and to recover *Candida* species (49,50) .

Interpretation of positive BCs

A key issue when interpreting a positive BC is to judge the reliability of the result. When a BC bottle is flagged as positive, four different scenarios can be considered, namely, true positive in either presence or absence of disease, contamination, or false positive. BC contamination (BCC) is defined as the growth of bacteria in BCs that are not present in

the patient's bloodstream. Contaminated BC can lead to unnecessary treatment, redundant laboratory testing, avoidable hospitalization, and an increased hospital stay; besides, the administration of unnecessary antibiotics increases selective pressure for antimicrobial resistance.

BCC is considered an indicator of healthcare quality and it should be kept as low as possible. According to the latest recommendations, it should not exceed 3% of the total BCs extracted with a target rate of 1% or even lower (51). The prevalence of BCC ranges from 0.6% to 17% (52) of all BCs performed, even though the figures vary from institution to institution. Microorganisms frequently associated with contamination include CoNS, viridans group streptococci, *Corynebacterium* spp., *Bacillus* spp., *Micrococcus* spp., and *Cutibacterium* spp.. Since some of these microorganisms (especially CoNS) are increasingly involved in true bacteraemia (e.g., patients with prosthetic devices or indwelling catheters), differentiating a contamination from a true pathogen remains challenging. Given the relevance of BCC in our institution, an entire section will be devoted to this matter.

Along with the patient's signs and symptoms, several parameters can be used to elucidate the role the isolated microorganism plays in infection. These include: 1) the type of microorganism isolated, 2) the number of positive bottles and the positivization pattern within a set, 3) sampling site: catheter versus venepuncture, and 3) TTP.

Identification and AST

The Gram stain is the initial test to perform for any positive BC. It is a quick, simple, and inexpensive test that helps characterize the microorganism involved. Indeed, the Gram

stain can help to assess the suitability of the selected antimicrobial therapy before definitive identification of the organism (53,54). Results of the Gram stain should be reported immediately to the physicians by telephone and by written report (55).

Species identification can be carried out by conventional biochemical methods, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), and/or nucleic-acid based methods. Introduction of MALDI-TOF MS has caused a revolution in clinical microbiology and has replaced conventional biochemical methods in many microbiology laboratories. Since its introduction in the clinical practice several preparation protocols have been described to directly identify organisms from positive BCs. Many of these protocols, commercially or laboratory designed, require haemolysis of red blood cells and several centrifugations for the separation of blood cells and microorganisms, and, therefore, can be tedious. More simply, MALDI-TOF performed after short incubation (2-5 h) on solid agar after inoculation with broth from a positive BC bottle has proved to be enough to identify successfully and reliably many of the growing microorganism. This technique is simple, efficient, cheap and does not require additional equipment, making it one of the most widespread (56).

Standard AST methods require 16–20 h of incubation. In the routine clinical practice AST is performed by automated systems that enable testing by broth microdilution a high number of antibiotics. Currently, there are three commonly used automated AST systems in our country: MicroScan Walkaway (Beckman Coulter), Vitek (bioMérieux) and Phoenix (Becton Dickinson). For the interpretation of the antimicrobial susceptibility results obtained by the aforementioned techniques, various committees such as the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial

Susceptibility Testing (EUCAST) publish and annually update breakpoint tables. Automated AST techniques are applied from grown colonies; the Phoenix and Vitek methods, which are the fastest, require a minimum mean time of 9 hours to obtain the results.

The more rapidly the appropriate antimicrobial is started, the lower the mortality for patients with sepsis. Ideally, the susceptibility profile of the organism is available at the time of therapy initiation, so that an appropriate antibiotic is chosen from the beginning. This is currently impossible, and antimicrobial therapy for acute infections is still initiated as empiric treatment. Published data suggest that rapid phenotypic methods with direct communication to the physician likely improve the timeliness of targeted antimicrobial therapy (57). With the current rates of multi-resistant microorganisms, the possibility of finding an infection caused by this type of microorganisms increases, which emphasizes the value of rapid AST (RAST). Antimicrobial treatment should be adjusted as soon as possible, and so, having a rapid and reliable AST plays a key role in the management of infection.

In current routine clinical practice, ASTs are only available the next day even though there are several technologies to accelerate ASTs or, at least, to detect specific resistant mechanisms. RASTs can be classified according to the time to result and to the clinical purpose (58). Buehler et al. (57) defined RASTs as technologies that provide results in $\underline{\leq}$ 8 hours, and ultra-rapid methods as the ones that yield results in ≤ 4 hours. Based on the clinical purpose, RASTs can be rapid single tests and accelerated full AST assays. Individual rapid tests involve additional costs and workload, as they are additional tests requested on demand that do not avoid having to perform the full AST. In contrast, accelerated full

AST assays provide a complete susceptibility report like the one provided by standard systems. However, the results of the entire antibiotic panel are usually not available until the afternoon or evening if started during the standard workday. The need for RASTs and, thus, shorter diagnostic turn-around times prompted EUCAST to develop a rapid and cheap phenotypic method based on disc diffusion directly from BC bottles. This method is easy to implement in any laboratory with extended operating hours. EUCAST defined for the first time in 2019 preliminary breakpoints for each of the reading times (4, 6 and 8h) for frequently isolated species in BC (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecalis* and *E. faecium*). A buffer area (area of technical uncertainty [ATU]) was defined between the susceptible and the resistant categories to reduce the appearance of false resistant and false susceptible results related to the technical variation accelerated by the short incubation time (59). Since then, the points have been periodically adapted.

As mentioned above, late identification of the causative pathogen species and its antimicrobial susceptibilities can often be responsible for delays in optimal antimicrobial therapy. Rapid molecular diagnostic tests, including tests such as polymerase chain reaction (PCR), MALDI-TOF, and peptide nucleic acid fluorescent in situ hybridization (PNA-FISH), have improved the microbiological diagnosis of BSI, reducing organism identification time and optimizing antimicrobial therapy, subsequently improving clinical outcomes, including mortality rates (60). This is so much so that the advancement of rapid diagnostic tests was included in 2015 among the five overarching goals of the National Action Plan to Combat Antibiotic-Resistant Bacteria during Obama's term (61).

However, the widespread implementation of these technologies has been limited due to inadequate outcome data and high costs.

Considering that many microbiology laboratories remain closed or do not have a microbiologist during the afternoon and/or night to validate and report the results obtained by accelerated systems, the speed achieved does not have an impact on the patient. This fact shows that the rapid availability of AST results is limited not only by technological reasons, but also by organizational aspects, the latter being at least as important (62). Therefore, it is particularly important to focus technological advances on those systems that allow AST results to be provided within the same work shift, so that they can be sent to physicians on the same day.

While it seems unlikely, at least in the near future, that full AST results are available in less than 4 hours, technically it is quite realistic to generate full AST results within 8 hours. Since sample processing, testing, validation, and interpretation take additional time, developing techniques that provide results in 6 hours would be especially advantageous. A recent study involving 209 laboratories in 25 European countries revealed that only 13% (25/192) of laboratories provided 24-h service to start immediate processing of BC bottles that have signalled positive, less than 5% of laboratories validated and informed results of identification and AST of microorganisms isolated in BC 24 h/day and 21.6% of laboratories did not inform BC identification or provide an AST on Sundays (63,64). This reflects those technological advances achieved in the diagnosis of BSI are seriously compromised by the limited operating hours of most microbiology laboratories.

In addition to improvements in existing conventional bacteraemia diagnostic techniques, there are currently new technologies for the diagnosis of bacteraemia, particularly those

that involve molecular techniques. Discussion of these technologies is beyond the scope of this document.

In any case, protocols of each hospital must be adapted to its own characteristics in relation to the available human and material resources, laboratory operating hours, type of patient and local resistance rates.

Epidemiology of BSI

Aetiology and site of infection of BSI

The total burden of BSI reported in a systematic review published in 2013 was of 575000–677000 episodes and 79000–94000 deaths per year in North America, and of over 1,2 million episodes of BSI 157000 deaths per year in Europe; BSI incidence ranged between 113 and 204 per 100,000 population (1). On the other hand, in 2017 an estimated 48.9 million (95% confidence interval [CI] 38.9–62.9) cases of sepsis, and 11.0 million (10.1–12.0) of sepsis-related deaths were reported worldwide, representing 19.7% (18.2–21.4) of all global deaths (64).

Knowledge of local epidemiology, aetiology, and susceptibility profiles is important to continually review and improve BSI diagnostic methods and treatment guidelines and to develop intervention strategies. The spectrum of microorganisms involved in BSI varies depending on the place of acquisition (CO, HO or HCA). Thus, while *S. pneumoniae* is a typical microorganism involved in CO bacteraemia, CoNS and *P. aeruginosa* are usually responsible for HCA infections. Differences may also exist between the resources of the setting: in high-income countries *E. coli*, *S. aureus*, *Klebsiella* spp., CoNS, and *P. aeruginosa* are amongst the most frequently isolated microorganisms in BSI, the first being the predominant pathogen in most settings. After *E. coli*, *Klebsiella* spp. is, generally, the second *Enterobacterales* species most frequently isolated. In low-income countries, *Salmonella enterica* may be part of the top ten pathogens involved in BSI.

Risk factors

Comorbidities including diabetes mellitus, chronic obstructive pulmonary disease, malignancies, hepatic cirrhosis, as well as immunosuppression following steroid therapy or antineoplastic therapy, are known risk factors for bacteraemia.

Blood culture contamination

BCs are essential for the diagnosis of BSIs and one of the most important tests performed in microbiology laboratories. Nevertheless, the prognostic value of BCs is limited by contamination (false-positive). A contamination is defined as the growth in culture of microorganisms that are not actually in the blood.

Contaminations represent up to 50% of positive BCs and can lead to unnecessary antibiotic treatment, redundant laboratory testing, avoidable hospitalization, increase in the patient's length of stay and unnecessary removal of lines. Therefore, contaminations should be minimized as much as possible. Guidelines recommend a contamination rate of < 3% (51), however recent studies show that this threshold is arbitrary and could be reduced. However, it should be borne in mind that regardless of what efforts are made to reduce or prevent contamination, a certain rate of BCs will inevitably be contaminated.

Although theoretically contamination can occur in any step, it typically occurs during the blood-sampling procedure, that is, during specimen collection, rather than during processing of bottles. Hence, the pre-analysis step of BC collection plays a significant role in BCC (65,66).

Prevalence and magnitude of the problem

Quantification of contaminated BCs can be done in two ways:

- overall contamination rate: calculating the percentage of contaminated BCs of all positive BCs.
- Calculating the percentage of contaminated BCs of all BCs obtained.

There is enormous variation in the reported BCC prevalence data. These variations, which range between 0.6% and 17%, are probably due, inter alia, to sampling conditions and to what each author considers contamination (52).

There is no "gold standard" to classify contaminants: some follow clinical criteria whereas others are guided strictly by microbiological criteria. BCs should be interpreted from a global patient perspective, so that to assess whether a BC is contaminated, several aspects must be considered: clinical findings and patient's condition judged by infectious disease physicians on the one hand, and microorganism involved and number of BC bottles and sets, among others, judged by microbiologists on the other. Thus, when comparing published data, contamination rates should be interpreted with caution.

Higher contamination rates have been uniformly communicated in EDs (both paediatric and ordinary) and are mainly due to lack of training, rapid staff turnover, workload and the patient's nature presenting in the ED. However, it should be borne in mind that in some institutions like ours most BCs are drawn in the ED.

Source of BCC

The potential sources of BCC are numerous and varied. Although contamination can occur during either specimen collection or processing of bottles, it is believed that it occurs practically always during specimen sampling (51). Contamination can be due to the transfer of microorganisms from the environment of the patient (or from the patient itself) o from the healthcare workers' hands or even saliva.

The main source of contaminants is commensal colonizing bacteria on the skin (such as CoNS) due to insufficient disinfection of the skin, studies having suggested that

contamination may be due to inadequate skin disinfection with reservoirs not reached by topical antiseptics. Therefore, poor technique by individuals obtaining BCs is a critical factor (66).

Drawing BCs through indwelling vascular catheters is related with higher contamination rates than those obtained by peripheral venepuncture, which is probably due to the difficulty in achieving adequate antisepsis in the port (i.e., access) area of the IV device at the site where blood is obtained for culture.

Other factors that were historically related to an increase in the rate of contamination were the use of BC bottles with media containing antibiotic-binding resins compared to standard broth media which increases the overall yield of both pathogens and contaminants (67,68), and the use of a single-needle technique compared to the two-needle technique of widespread use before the human immunodeficiency virus (HIV) era (69).

Methods for classification of contaminants

To assess whether a BC is contaminated, several aspects should be considered: clinical findings and patient's condition determined by infectious diseases physicians on the one hand, and type of microorganism involved and number of positive BC bottles and sets, among others, judged by microbiologists on the other. Even following both clinical and microbiological criteria, the interpretation of positive BCs for CoNS is often troublesome.

Identity of microorganism

The identity of the microorganism isolated is one of the most important factors that influences the clinician's decision. While certain microorganisms almost always represent true pathogens when isolated, other microorganisms are frequently considered contaminants unless recovered from multiples BCs obtained in sequence. These include CoNS, *Corynebacterium* spp., *Cutibacterium* spp., *Bacillus* spp., and *Micrococcus* spp.. Nevertheless, some of these microorganisms, especially CoNS, are increasingly involved in true bacteraemia: central venous line-associated infection, prosthetic joint infection, endocarditis, infection related to foreign devices, among others. Therefore, differentiating a contamination from a true bacteraemic episode is challenging and can not only relay on the microorganism involved. Enterococci, streptococci of the viridans group and *Clostridium* spp. are of variable clinical importance, and microorganisms such as *S. aureus*, *S. pneumoniae*, beta-haemolytic streptococci, *Listeria monocytogenes*, *E. coli* and other *Enterobacterales*, *P. aeruginosa*, *N. meningitidis*, *H. influenza*, and *Candida* species rarely represent contamination (Table 4) (52).

The clinical microbiology laboratory plays a fundamental role in providing instructions and developing updated specimen collection guidelines for preventing contamination during BC extraction. Moreover, it plays an essential role in providing information to care providers when BC isolates that likely represent contamination are recovered.

Table 4. Likelihood of representing contamination (unless recovered from multiple BCs).

RARELY REPRESENT CONTAMINATION	VARIABLE CLINICAL SIGNIFICANCE	LIKELY REPRESENT CONTAMINATION
- *S. aureus* - *S. pneumoniae* - β-haemolytic streptococci - *L. monocytogenes* - *E. coli* - *Enterobacterales* - *P. aeruginosa* - *N. meningitidis* - *H. influenzae* - Anaerobic Gram-negative rods (*Bacteroides* spp., *Fusobacterium* spp.) - *Candida* spp.	- Enterococci - *Clostridium* spp. - Viridans group streptococci	- CoNS - *Bacillus* spp. - *Corynebacterium* spp. - *Cutibacterium* spp. - *Micrococcus* spp.

CoNS: coagulase-neagtive staphylococci.

Number of positive BC bottles within a set and TTP

It is well known that increasing the number of positive bottles within a set helps predicting the likelihood of true bacteraemia, especially when a CoNS are isolated (52). So, when a CoNS or other potential contaminant is isolated from one or two bottles belonging to the same BC extraction it is likely to be considered as a contaminant.

On the other hand, the amount of time required for the organism to grow is another determining factor. It has been hypothesized that BCs from a true bacteraemia will have a higher inoculum of bacteria and consequently will grow faster than a contaminated

culture. Nonetheless, there are factors that may reduce the accuracy of this parameter liker prior administration of antimicrobials, BC volume, delay, or sample transfer, among others.

Phenotyping, genotyping profile

Comparing susceptibility profiles has routinely been used to elucidate the relatedness of strains. Nevertheless, it has been demonstrated that strains sharing the same antibiotype may have in fact different genotypes (70). Although pulsed-field gel electrophoresis has proven to be a powerful tool for distinguishing contaminant from pathogen CoNS, this technique, along with other molecular typing techniques, is impractical in daily clinical practice.

Consequences

Clinical consequences

Contaminants have direct consequences on the patient, as they can result in:

- unnecessary or prolonged antibiotic treatments which is associated with the consequently selection of resistant strains and with several potential adverse events, including allergic reactions, drug interactions, disruption of the host microbiome that can lead to *Clostridioides difficile* infection as well as other adverse consequences. On the other hand, for the administration of antimicrobials, venous access must be maintained, which can result in mechanical

complications, thromboembolic disease, and infection. In the study by Souvenir et al. 41% (out of 59 patients in the contamination category) of BC contaminant episodes due to CoNS were treated with antimicrobial agents (71). Lee et al. found that of 178 cases with pseudobacteraemia, 41 % were unnecessarily treated with IV antibiotics, of which glycopeptides accounted for 20% (72). In addition, many of the patients who were started on antibiotics for contamination events received prolonged therapy, mean duration of 6.5 days (71) or 7 days (73).

- Prolonged hospital stay. An estimated 15 surgical procedures will be postponed annually due to contamination events, which may lead to an increase in lengths of stay (51,74). Gander et al. found that for patients with BCs obtained in the ED prior to admission the median length of stay increased from 4 to 5 days, and among hospitalized patients (75), Alahmadi et al. demonstrated that contaminated BCs were associated with a 5.4-day increase in hospital stay compared with that of hospitalized controls matched for age, comorbidity score and month of admission to the hospital (76).
- In turn, each additional day of hospitalization due to BCC increases the chances of a hospital-acquired adverse event, nosocomial infections, medication errors, or falls, pressure ulcers and thromboembolic events, depending on the patient's condition and the severity of the disease.
- Additional diagnostic tests such as echocardiography or new batch of BCs, and unnecessary consultation requests. Searching for a source of bacteraemia can lead to unwarranted concern or even removal of permanent devices such as pacemakers or implantable cardioverter-defibrillators.

- The initial focus on the BC result as the aetiology of the patient's clinical syndrome may result in "anchoring bias" (a form of cognitive bias in which one relies too heavily on initial data when making later decisions) which can lead to a delay in obtaining the correct diagnosis and a delay in initiating appropriate therapy (51).

Economic consequences

Although the economic impact is linked to the clinical impact (prolonging hospital stay, increasing the number of complementary diagnostic tests, additional antibiotic treatment …) will be treated as an independent section due to the significance it has in an increasingly weakened system.

Geisler et al. estimated in a recent retrospective observational study a cost per BCC event of up to $6,463. In this study, 79% of the increased cost of care was the result of increased length of stay and increased duration of antimicrobial therapy (77).

Several investigations have evaluated the economic benefits of routine use of interventions to reduce costs associated with BCC. Thus, Self et al. compared overall hospital costs associated with three collection strategies: usual care, sterile kits, and phlebotomy teams. Compared with the routine BC collection procedure, annual net savings using the sterile kit and phlebotomy team strategies were $483,219 and $288,980, respectively (78). On the other hand, Skoglund et al. evaluated the potential clinical and economic benefits of an initial specimen diversion device when routinely used for BC collection in the ED. It was estimated that the routine use of the diversion device

to prevent contamination would result in overall hospital cost savings of $272 per BC obtained in the ED (79).

Preventing contamination

This section will examine all aspects to consider to minimize the number in the contamination rate.

Patient selection

Prevention of BCC begins with selecting the right patients to draw BC from and thus avoiding opportunities to isolate contaminants. The pre-test probability of bacteraemia has an important impact on the positive predictive value of the BC result. Thus, the extraction of BCs in patients with a very low probability of bacteraemia results in positive cultures that are more likely to represent false positives. Therefore, a good education of physicians on the clinical conditions most frequently associated with bacteraemia is necessary (51).

As stated before, although there is no universal recommendation on what the indications of BCs are, BCs should be drawn in the presence of chills, fever (body temperature 38 °C) or hypothermia in neonates and elderly patients. BCs are also warranted whenever leukopenia, leucocytosis or thrombopenia that are not related to haematological processes are present, in the presence of other signs of focal infection or sepsis, as well as in the case of suspected endocarditis. In addition, they should always be drawn when a catheter tip is removed and sent to culture in case of suspected catheter-related bacteraemia. Moreover, BCs should be performed in patients with suspected meningitis, osteomyelitis, pyelonephritis, intra-abdominal infection, arthritis, severe skin and soft

tissue infections, pneumonia, endocarditis, and fever of unknown origin (abscess, typhoid fever, brucellosis, tularaemia, etc.). BC extraction is also indicated in young or elderly children with sudden decay, as typical signs, and symptoms of bacteraemia may not occur in these populations. BC should be supplemented whenever possible with samples from other locations to try to determine the focus of the process.

Skin preparation and disinfection

The patients' skin at the venepuncture site is thought to be the most frequent source of contamination. As early as 1972, Selwyn and Ellis (65) showed that about 20% of skin bacteria live within deep layers of the skin protected by follicles, crevices, or lipids so that topical antiseptics cannot efficiently penetrate.

Which antiseptic performs better for skin disinfection is still a matter of debate. Alcohol, chlorhexidine, and iodine products (iodine tincture, povidone-iodine and iodophor) are the most universally used agents. For skin preparation prior to venepuncture alcoholic products seem to be superior to non-alcoholic ones in preventing BCC. Data from the literature do not support a preference for alcoholic chlorhexidine over an alcoholic preparation containing iodine, alcohol followed by another disinfectant, or even alcohol alone (80,81). The most widespread guideline is to disinfect the phlebotomy site with 2% alcoholic chlorhexidine, or 70% isopropyl alcohol followed by 2% chlorhexidine, allowing at least 30 seconds to dry. For infants < 2 months of age, alcohol swabs should be used rather than chlorhexidine (66). Nevertheless, as there are several valid options available, the choice of disinfectant may vary depending on each hospital.

It should be borne in mind that the action of antiseptics is not immediate so the appropriate time interval between their use and the puncture must be respected (approximately 30 seconds in the case of chlorhexidine and between 1.5 and 2 minutes if povidone is used).

A good technique to disinfect the skin is believed to be even more important than the agent used. As for the method of application, it is traditionally recommended to perform circular movements working out from the intended needle-insertion site whenever aqueous-based products are used to prevent the reintroduction of contaminants in the cleansed area. However, a more recent study concludes that there is insufficient data to justify this technique when alcoholic solutions are used and consider vigorous friction to be more effective (82).

BC bottle tops disinfection

The rubber stopper of each bottle of BC, despite being covered with a cap that must be removed prior to inoculation, is not sterile. Therefore, although data is sparse, it is standard practice to disinfect the tops of BC bottles before inoculating with blood. The antiseptic used to clean the cap should be allowed to dry, thus preventing it from entering the bottle when inoculating the blood and preventing possible inhibition of bacterial growth.

In this process, iodine alone should be avoided, as it can cause erosion of the rubber plug during incubation, introducing contaminants (83).

BC collection site: venepuncture vs. catheter draw

It is preferable to obtain blood for culture by venepuncture from veins in the antecubital fossae rather than through indwelling vascular access lines, except in patients with suspected line-related infections, where blood should be drawn through the line as well as from venepuncture sites, and the results of both should be compared to help identify line-related infections. A meta-analysis including nine studies showed that blood collected through an intravascular catheter had, on average, a 2.69-fold greater likelihood of being contaminated than blood collected via venepuncture (95% CI, 2.03 to 3.57) (84).

Pre-packaged collection kits

The impact of using pre-packaged collection kits in BCC is not entirely clear. Although in some studies the use of these kits has been associated with a decrease in BCC (85-87), a meta-analysis of seven studies showed that the use of pre-packaged collection kits did not result in a significant reduction in the BCC rate (84). The effect of sterile drapes on BCC has not been studied in isolation, but sterile drapes are usually part of pre-packaged collection kits.

Sterile Gloves and Hand Hygiene Sterile

The use of sterile gloves for BC extraction has been an element in numerous multi-component improvement projects for BCC reduction as nonsterile gloves can become contaminated and result in BCC. In a single-centre crossover trial the use of sterile gloves was associated with a significant decrease in BCC (88). In any case, sterile gloves should be used whenever it is necessary to palpate or re-palpate a venepuncture site after it has been disinfected.

Hand hygiene is universally recommended before contact with any patient as a standard infection prevention. Nevertheless, there is little data to suggest that hand hygiene influences in the contamination rates of BC (51).

Phlebotomy team

The use of trained phlebotomists or specifically educated nurses to obtain BCs has been associated with decreased BCC rates (89,90).

Sampling and Volume

Sampling an adequate volume of blood is considered the most important parameter for the microbiological diagnosis of BSI and is essential in optimizing the performance characteristics of BCs. Both underfilling and overfilling BC vials have been associated with contamination and/or false-positive results (91). As mentioned before, for adults, the target volume is standardized in 8-10 mL per bottle, that is, 16-20 mL per BC, and in children the volume depends on age and/or weight of the patient. The practice of obtaining multiple blood samples during a single phlebotomy is discouraged.

Educational intervention

The implementation of educational interventions to reach both nurses and physicians has been shown to reduce the rate of BCC (92). Likewise, numerous studies have shown that surveillance for BC, most commonly using laboratory parameters for the definition of contamination, and feedback systems improve BCC rates, especially when the results are informed on an individualized basis to those who perform BC extraction.

Initial Specimen Diversion

One approach to reduce BCC is to avoid culturing the first portion (0.5-2 mL) of blood, which can contain incompletely sterilized fragments of skin from the needle stick and presumably bacteria, with culture of the remaining secondary aliquot of blood.

When applied by trained phlebotomists, initial-specimen diversion techniques either using sterile vacuum blood collection tubes or a commercially available designated initial specimen diversion device, have demonstrated a reduction of 30% to 50% in BCC (93–95). An advantage of the approach proposed by Lalezari et al. which proposes the diversion into collection tubes, is that the blood can be used for other tests that are commonly ordered for patients with suspected BSI in addition to not incurring additional costs (95).

Table 5 summarizes the interventions that can be implemented to prevent BCC.

Table 5. Interventions to prevent BCC. *Adapted from Doern et al.* (51)

INTERVENTION	COMMENT
Patient selection	Select the right patients from which to draw BC.
Skin disinfection	▪ Use of an alcohol containing disinfectant. ▪ Infants < 2 months of age: alcohol swabs should be used rather than chlorhexidine. ▪ Allow skin antiseptic to dry fully: • chlorhexidine: at least 30 seconds. • Iodophors: at least 1.5 and 2 minutes. ▪ Vigorous friction.
Phlebotomy site	▪ Venepuncture from veins in the antecubital fossae. ▪ Avoid drawing BC via intravascular catheters unless the catheter is thought to be the source of bacteraemia.
Sterile gloves/hand hygiene	▪ Limited data to support sterile gloves use; they should be used whenever it is necessary to re-palpate a venepuncture site after it has been disinfected.
Standardized kits	▪ Limited data available.
Volume	▪ Adults: 8-10 mL per bottle (16-20 mL per BC). ▪ Children: depends on age and/or weight of the patient. ▪ The practice of obtaining multiple blood samples during a single phlebotomy is discouraged. ▪ Under- and overfilling BC bottles may lead to contaminants/false positive results.
Phlebotomy team/education	▪ Proven useful in decreasing BCC in numerous studies.
Initial specimen diversion	▪ Sterile vacuum blood collection tubes or commercially available designated initial specimen diversion devices: have shown promising results in reducing BCC.

BC: blood culture, BCC: blood culture contamination.

HYPHOTHESES AND OBJECTIVES

HYPOTHESES

1. The increasing age of the patients cared for, and the greater number of immunosuppressed patients and the risk associated with invasive procedures to which the patients are subjected may have caused a change in the aetiology of bacteraemia and the antimicrobial susceptibility profiles of microorganisms involved in BSI in recent years.
2. The implementation of rapid antibiograms in the Microbiology Department makes it easier for the clinician to have early antibiotic therapy adjusted to the susceptibility of the microorganism.
3. The implication of the causative microorganism or contaminating microorganisms could be deduced from the TTP of BCs.
4. The SARS-CoV-2 pandemic may have led to a change in the use and performance of BCs in the hospital.

OBJECTIVES

The objectives of this doctoral thesis have been:

1. Evaluate trends in workload, incidence, aetiology, and resistance profiles to key antibiotics in BSI over a six-year period (2015-2020) in both a tertiary and a district hospital.

2. Evaluate two assays using E-tests and VITEK®-2 for rapid detection and accurate differentiation of bacterial antibiotic susceptibility in *Enterobacterales*.

3. Analyse the TTP of BCs during a two-year period.

    a) Evaluate the TTP of all bacteraemia episodes (true and contaminations) isolated during a two-year period (2019-2020) to study to what extent the TTP can provide information on the type of microorganism isolated, and its involvement in infection.

    b) Analyse those episodes in which the TTP was greater than 24 h.

    c) Analyse separately all candidaemia episodes and determine the relationship between the TTP and 28-day mortality.

    d) Explore the influence of the TTP and the positive bottle detection pattern (PBDP) in BCC by CoNS.

4. Evaluate the impact of the SARS-CoV-2 pandemic on BC utilization and describe the characteristics of bacteraemia in SARS-CoV-2 patients.

HIPÓTESIS

1. El aumento de la edad de los pacientes atendidos y el mayor número de inmunodeprimidos o sometidos a tratamiento inmunosupresor y el riesgo asociado a los procedimientos invasivos a los que son sometidos los pacientes puede haber ocasionado un cambio en la etiología de la bacteriemia y los perfiles de sensibilidad a los antimicrobianos de los microorganismos implicados durante los últimos años.
2. La incorporación de los antibiogramas rápidos a la cartera de servicios del Laboratorio de Microbiología facilita que el clínico disponga de una antibioterapia precoz y ajustada a la sensibilidad del microorganismo.
3. La implicación del microorganismo causal de la bacteriemia o los microorganismos contaminantes podría ser deducida del tiempo de positivización de los hemocultivos.
4. La pandemia de SARS-CoV-2 ha podido suponer un cambio en la utilización y el rendimiento de los hemocultivos en el hospital.

OBJETIVOS

Los objetivos de esta tesis doctoral han sido:

1. Evaluar las tendencias en la carga de trabajo, la incidencia, la etiología y los perfiles de resistencia a antibióticos clave en bacteriemia durante un período de seis años (2015-2020) tanto en un hospital terciario como en uno comarcal.

2. Evaluar dos métodos de antibiograma rápido utilizando E-tests y VITEK®-2 para una detección rápida y una diferenciación precisa de la sensibilidad antimicrobiana en enterobacterias.
3. Analizar el tiempo de positivización de los hemocultivos durante un período de dos años.
    a) Evaluar el tiempo de positivización de todos los episodios de bacteriemia (verdaderos y contaminaciones) aislados durante un período de dos años (2019-2020) para estudiar en qué medida el tiempo de positivización puede aportar información sobre el tipo de microorganismo aislado y su implicación en la infección.
    b) Analizar aquellos episodios en los que el tiempo de positivización fue superior a 24 h.
    c) Analizar por separado todos los episodios de candidemia y determinar la relación entre el tiempo de positivización y la mortalidad a los 28 días.
    d) Explorar la influencia del tiempo de positivización y el patrón de positivización de los frascos en episodios contaminados por estafilococos coagulasa-negativos.
4. Evaluar el impacto de la pandemia de SARS-CoV-2 en la utilización de hemocultivos y describir las características de la bacteriemia en pacientes con SARS-CoV-2.

MATERIAL AND METHODS

GENERAL MATERIAL AND METHODS

Setting

The study was conducted at the core Microbiology Laboratory of the University Hospital of Araba (HUA), a centralized clinical microbiology laboratory belonging to the Spanish National Health System.

The core Microbiology Laboratory provides service to patients receiving care at a network of hospitals:

- the HUA: tertiary and interdisciplinary, which comprises the Txagorritxu Hospital and the Santiago Apóstol Hospital. Both hospital headquarters are located at Vitoria-Gasteiz (Araba) and are approximately 3.8 km from each other. The Txagorritxu hospital is 500 m from the Microbiology Laboratory and the Santiago hospital 2.6 km.
- The Hospital of Alto Deba (HAD): district hospital located in the Alto Deba region (Gipuzkoa). The hospital is about 38 km from the Laboratory.
- The Hospital of Leza: rural hospital located in the municipality of Laguardia (Araba) 43 km from the Laboratory.

Given the small number of samples belonging to the Hospital of Leza, these will be included within the HUA.

The characteristics of the HUA and the HAD are shown in Table 6. The HUA supports a population of about 340000 inhabitants, mean annual hospital admissions were 40152 during the study period, and has approximately 734 standard hospital beds and 30 ICU beds. On the other hand, the HAD provides care to around 65000 inhabitants, mean annual hospital admissions were 5471, and has about 74 beds.

Table 6. General parameters of the HUA and of the HAD.

				2015	2016	2017	2018	2019	2020
HUA	▪ Type: tertiary hospital. ▪ Population supported: 340000. ▪ Hospital system type: multiple institution system. ▪ ASP (antimicrobial stewardship program): yes.	Total	Hospital admissions	39932	40205	40714	42100	41505	36458
			Average length of stay	5.05	5.01	4.90	4.70	4.82	5.03
			Hospital stays	201526	200874	199507	198476	200231	183921
			Number of beds	707	705	724	748	745	776
			Number of ICU beds	30	31	31	26	31	31
			ED consultations	164047	168757	169095	171393	176078	125156
			ED hospital admissions (%)	21426 (13.1)	20914 (12.4)	20838 (12.3)	21569 (12.6)	21042 (12.0)	18454 (14.7)
		Children	Hospital admissions	744	716	672	787	699	557
			Average length of stay	4.28	4.47	4.08	4.3	3.78	3.12
			Hospital stays	3216	3177	2737	3390	2596	1.778
			Number of beds	15	20	20	20	20	20
HAD	▪ Type: district hospital. ▪ Population supported: 65000. ▪ Hospital system type: single institution. ▪ ASP: no.	Total	Hospital admissions	5945	5582	5606	5581	5425	4686
			Average length of stay	3.43	3.42	3.39	3.21	3.15	3.28
			Hospital stays	20514	18992	18938	17960	17060	15377
			Number of beds	76	72	72	73	72	77
			ED consultations	39315	41819	40868	40453	41269	29731
			ED hospital admissions (%)	3232 (8.2)	3095 (7.4)	3081 (7.5)	3087 (7.6)	2934 (7.1)	2587 (8.7)

HUA: University Hospital of Araba, HAD: Hospital of Alto Deba, ICU: Intensive care unit, ED: Emergency Department.

Study design

Objective one: the study design was a descriptive and retrospective cohort design; no interventions were planned.

Objectives two, three and four: the study design was descriptive and prospective.

The study received approval by the Ethical Committee for Clinical Research of the Basque Country (PI2021144).

Patients

Patients included in this study were those who had BCs drawn between January 2015 and December 2020 in both the HUA and the HAD.

Preanalytical phase

The preanalytical phase comprises test selection, patient identification, sample collection, sample handling, and sample transport.

Sample collection

The extraction of the blood to be inoculated in BC bottles was carried out following the protocols available for this purpose in both hospitals. These provide information about the time and anatomical sites of extraction, optimal volume of blood to be obtained, the atmosphere of the culture bottles, number of extractions and aseptic conditions that must be followed and are periodically reviewed. The periodic review of these protocols

is a task carried out by the Microbiology Department, the Preventive Medicine Department and the hospital's nursing staff.

The extraction of blood is carried out by the nursing staff of each department. At our hospital there is no dedicated team of phlebotomists who routinely perform BC collection.

The extraction is usually performed by venepuncture (peripheral extraction) from veins, avoiding extraction from intravascular devices. In certain departments (e.g., ICU, Oncology Department) and in certain patients (e.g., when line-related infections are suspected), blood is drawn from central lines, Hickman lines, arterial lines, or other lines. Whenever this technique is performed, protocols available strongly recommend collecting these samples at the same time as a peripheral BC, and carefully labelling bottles with the site and time of collection.

The general practice of collecting at least two sets each containing one aerobic and one anaerobic BC bottle, from different peripheral sites, is widespread in both hospitals. Only in paediatric patients or in patients in whom blood collection is difficult blood is inoculated into a specific paediatric flask or into an aerobic BC bottle.

For adults, the target volume is standardized in 8-10 mL per bottle, that is, 16-20 mL per BC, and in children the volume depends on age and/or weight of the patient.

Conservation, transport, and processing of the sample

As mentioned above, the core Microbiology Laboratory at the HUA provides service to patients receiving care at a network of hospitals: the Txagorritxu Hospital, the Santiago Apóstol Hospital, the HAD, and the Hospital of Leza. Three satellite BC incubators operating 24/7 (24-h, 7-day/week) are installed closed to the EDs of the most important hospitals belonging the network: one in the Txagorritxu Hospital, another in the Santiago Apóstol Hospital and a third incubator in the HAD. In addition, the Microbiology Department itself has its own incubator.

The Microbiology Laboratory operates from 8:00 to 15:00 from Monday to Saturday. Positive BC bottles are sent in two (HUA) or a single shift (HAD) to the Laboratory. Additionally, in exceptional situations, additional transports may be requested.

Analytical phase

The analytical phase consists of all the processes involved in testing of a sample. As mentioned in the Introduction section, errors occur much less frequently in the analytical phase than in the preanalytical or postanalytical phases.

BC media

BC bottles used throughout the study were BD BACTEC bottles (Becton Dickinson): BD BACTEC™ Plus Aerobic medium vials, BD BACTEC™ Lytic Anaerobic medium vials, and BD BACTEC™ Peds Plus™ Medium vials.

Incubators

Incubation of BCs was carried out in BD Bactec FX automated instruments (Becton Dickinson). BC bottles contain a sensor which responds to the concentration of CO_2 produced by the metabolism of microorganisms, or the consumption of oxygen needed for the growth of microorganisms. The sensor is monitored by the instrument every ten minutes for an increase in its fluorescence, which is proportional to the increasing amount of CO_2 or the decreasing amount of O_2 present in the flask. Positive cultures are immediately flagged by an indicator light on the front of the instrument, an audible alarm, and are displayed on the LCD display.

When positive vials are identified, the lab technician pulls them from the instrument for confirmation of results.

Incubation period

The usual maximum incubation period until a BC was considered negative was of five days. In certain circumstances, the incubation lasted up to fourteen days or more, at the discretion of the physician in charge: suspicion of endocarditis, fungal infection, or other slow-growing microorganisms.

Species identification

BC samples were processed following standard microbiological procedures.

Species identification was carried out either by biochemical panels by MicroScan (Beckman Coulter) or by API strips (BioMérieux) until January 2016. From that date on, identification was performed by MALDI-TOF, MALDI Biotyper system (Bruker Daltonics).

Scores above 2 were considered valid. 16S rRNA gene sequencing was performed whenever MALDI-TOF did not provide appropriate results.

Since 2017, to speed up the identification of microorganisms by MALDI-TOF MS, a short incubation protocol was implemented based on species identification from immature biomass growing on solid media that consisted of:

- two drops of positive BC broth were plated on a Chocolate agar plate, each at one end of the plate. One was spread over the plate surface with an inoculation loop, the other was not. Then, plates were incubated 5% CO_2 and 36±1 °C.
- Chocolate plates inoculated with material from the positive BC bottle were visually evaluated for the first time 2-3 h after start of incubation.
- As soon as growth of biomass (rather mature “colonies”) was visible on the agar plates, species identification was performed from these “young” cultures using MALDI-TOF MS.
- If the species identification result was available with appropriate scores (scores above 2), it was reported. If species identification was achieved but with a low score, the result was provisionally reported. If no growth was visible, or no successful identification was achieved, the next visual evaluation was performed after further 2-3 h of incubation. If enough growth was not achieved during the morning shift, the identification was repeated the next day, after 24 hours incubation.
- In general, if the identification carried out by the rapid method was conclusive, it did not need to be repeated after 24 hours. However, and especially for Gram-

positive bacteria, the identification had to be confirmed after 24 hours of incubation.

Since July 2017, serotypes of *S. pneumoniae* isolates were determined whenever possible (135) using a reverse-hybridization test (S.PneumoStrip®, Operon).

AST

AST was carried out by standard broth microdilution method using dehydrated panels manufactured by MicroScan (Beckman Coulter).

For streptococci, *Haemophilus* spp., *Candida* spp. and anaerobes Minimum Inhibitory Concentrations (MICs) were determined by Sensititre® MIC susceptibility plates (Thermo Scientific).

MICs using the E-test method (BioMérieux) were used to perform verifications and to test additional antibiotics, especially in resistant strains.

The number of antibiotics tested varied depending on the bacterial species and the standard panel used, which usually includes the most common antibiotics.

AST was interpreted following the EUCAST guidelines of 2021 in the case of bacteria, and the 2020 EUCAST guidelines for interpretation of MICs for antifungal agents in the case of yeasts. For enterococci and daptomycin, epidemiologic cut-off values (ECOFF) were followed.

Extended-spectrum beta-lactamase (ESBL) production was detected using the double disk diffusion test and more recently the MIC Test Strip-ESBL strips (Liofilchem)

(cefotaxime/cefotaxime-clavulanate, cefazidime/ceftazidime-clavulanate and cefepime/cefepime-clavulanate) specifically designed to confirm the presence of clavulanic acid inhibitable ESBL in *Enterobacterales*. Carbapenemase was detected and confirmed using the immunochromatographic (ICT) test CARBA-5 (NG biotech), which targets KPC, OXA-48 and OXA-48 like, VIM, IMP and NDM carbapenemases and a multiplex PCR with targets for VIM, KPC, OXA-48, IMD, NDM (RealCycler, Progenie molecular).

Definitions

The following terms were defined prior to data analysis.

- A BC was defined as aerobic and anaerobic BC bottles obtained from one venepuncture site.
- Children were those patients under 14 or 14 years of age.
- Polymicrobial episodes were considered those in which two or more clinically significant microorganisms were isolated from the same BC bottle or from different bottles belonging to the same BC set or within 48 h of each other.
- Isolates were classified as non-resistant or resistant; non-resistant isolates were those that were either susceptible or intermediate according to 2021 EUCAST guidelines.
- Following multi-drug resistance (MDR) indicators of the PIRASOA program (96), based on the definitions of multi-resistance for *P. aeruginosa* and *A. baumannii* of the German Society for Hygiene and Microbiology (97), *P. aeruginosa* isolates were classified as MDR if they were only susceptible to one or none of the

following antibiotics: piperacillin-tazobactam, ceftazidime, meropenem, and ciprofloxacin.

Data analysis

Data analysis for each objective will be detailed in the next section.

In brief, we expressed categorical variables as counts (percentage) and continuous variables as mean and standard deviation (SD) or median and interquartile range (IQR), where appropriate.

Comparison of two proportions was performed using the Fisher exact test with two tails. Significance was assessed at $P<0.05$.

OBJECTIVE-SPECIFIC MATERIAL AND METHODS

Objective 1: evaluate trends in workload, incidence, aetiology, and resistance profiles to key antibiotics in BSI over a six-year period (from 2015 to 2020) in a tertiary and a district hospital.

Study design

BC data obtained from January 2015 to December 2020 was retrieved from the electronic database of the centralized Microbiology Laboratory of the HUA.

The computer system with which data extraction was carried out was GestLab (launched in mid-2019) and Whonet. Additional consultations were made on systems used in previous years such as Omega.

Patients

Patients included in this study were those who had BCs drawn between January 2015 and December 2020 in both the HUA and the HAD. As mentioned earlier, patients belonging to the Hospital of Leza were included in the HUA.

Laboratory methods and sample processing

Previously discussed in the analytical phase section.

Data analysis

Data were analysed globally, and trends were calculated. Likewise, BCs requested in ED were separately analysed.

Given the low number of paediatric BSI episodes isolated in HAD, the number of episodes in that hospital was provided as a whole, not breaking down paediatric and adult patients.

Statistical analysis

Linear regression analysis was applied to determine trends. Comparison of two proportions was performed using the Fisher exact test with two tails. Significance was assessed at $P<0.05$.

Objective 2: evaluation of two assays using E-tests and VITEK®-2 for rapid detection and accurate differentiation of bacterial antibiotic susceptibility in *Enterobacterales*.

Study design

A total of 121 prospective BC positive samples isolated from March to December 2019 showing Gram-negative enteric bacilli, derived from patients suspected of having BSI, were included in the study.

Polymicrobial cultures were excluded from the evaluation.

Only those episodes for which it was feasible to perform the RAST on the same day BCs had become positive were included.

Patients

Patients included in this study were those with suspected bacteraemia caused by *Enterobacterales*.

Laboratory methods and sample processing

Once a BC was found to be positive and Gram stain revealed Gram-negative bacilli compatible with *Enterobacterales*, an aliquot was used to perform the AST with E-test strips, another to inoculate the VITEK®-2 cards using the direct test procedure, and another aliquot was subcultured on a combination of agar plates to carry out the identification of the microorganism and the AST in a conventional way.

Direct susceptibility testing by E-test was performed by applying four to six drops of the positive BC broth to Mueller-Hinton agar plates. A sterile cotton swab was then used to inoculate each plate by rubbing the swab over the entire surface of the plate in three directions, rotating the swab while inoculating. The following antibiotics were evaluated: amoxicillin-clavulanate, piperacillin-tazobactam, cefotaxime, meropenem, ciprofloxacin, and amikacin. The plates were then incubated in 35±1 °C. Antibiotics MICs were read after five and seven hours of incubation and data were recorded.

To inoculate the VITEK®-2 cards directly from a positive BC vial, 5-6 drops from the BC bottle were inoculated into the VITEK®-2 inoculation vial containing 3 mL 0.45% saline solution. It was then vigorously vortexed for at least 30 seconds and immediately processed in a conventional way (following the manufacturer's instructions).

It was recorded for which antimicrobials the MIC was available after five and seven hours.

Since the panels compared (MicroScan 52 and VITEK®-2 AST-N243) had a different selection of antibiotics, the comparison was focused on those antibiotics that are included in both test panels and that are often used in the treatment of BSI: amoxicillin-clavulanate, piperacillin-tazobactam, cefotaxime, ceftazidime, cefepime, ertapenem, meropenem, imipenem, ciprofloxacin, gentamicin, and amikacin.

Direct AST results were compared with the results obtained from the reference method MicroScan (Beckman Coulter). The reference method results were used as gold standard for AST testing. The results of standard AST were not known to the examiner of direct AST.

Comparison between early reading and standard incubation was performed for each bacteria/antibiotic combination, based on SIR categorisation.

Errors were categorised as minor errors (mE), major errors (ME) and very major errors (VME) (Table 7).

In the event of insufficient growth to read MICs, not readable (NR) was scored. Likewise, NR was scored whenever MICs could not be determined due to insufficient growth during the run time of the VITEK 2 analysis (indicated as terminated results [TRM]).

Table 7. Definitions of error type.

Minor errors (mE)	Erratic assignment to adjacent interpretative categories (susceptible to intermediate, intermediate to susceptible, intermediate to resistant, resistant to intermediate).
Major errors (ME)	Erroneous categorization of true-susceptible isolates as resistant.
Very major errors (VME)	Erroneous categorization of true-resistant isolates as susceptible.

During the validation process, the results were not reported to the clinician.

Categorical agreement (CA) was evaluated.

Data analysis

Comparison of test results was performed on a categorical level.

Objective 3: i) evaluate the TTP of all bacteraemia episodes (true and contaminations) isolated during a two-year period (2019-2020) to study to what extent the TTP can provide information on the type of microorganism isolated and its involvement in infection, ii) analyse those episodes in which the TTP was greater than 24 h, iii) analyse separately all candidaemia episodes and determine the relationship between the TTP and 28-day mortality, and iv) explore the influence of the TTP and the PBDP in BCC by CoNS using BLR analysis and achieve a predicting formula.

Study design

From January 2019 to December 2020, all episodes were prospectively recorded. Of them, 1893 were considered true (1778 monomicrobial, 115 polymicrobial) and 1106 contaminations, attending to both clinical and microbiological criteria.

Episodes categorized as real but considered doubtful, or inconclusive were excluded from the study. Follow-up BCs were not considered. After an exhaustive retrospective review of each episode and of the corresponding medical records, 109 episodes were discarded from the analysis.

TTP was defined as the time from the start of incubation to a positive signal in the Bactec FX continuous monitoring system and was calculated automatically.

TTP of the first positive bottle of all episodes was recorded and evaluated.

Polymicrobial episodes were excluded from the evaluation of the TTP by type of microorganism since the TTP of individual microorganisms remains unknown in these cases.

Those microorganisms that were not included in the following categories accounted for 2.9% of the true episodes and were considered rare: *Enterobacterales*, non-fermenting Gram-negative bacilli (NFGNB), *S. aureus*, CoNS, *Streptococcus* spp., *Enterococcus* spp., strict anaerobes, and *Candida* spp.

Laboratory methods and sample processing

Previously discussed in the analytical phase section.

In 2019, mean volume per flask was of 9.0 mL (95% CI, 8.1-9.8, SD 3.9 mL) and in 2020 of 8.5 mL (95% CI, 7.7-9.4, SD 4.0 mL).

PBDPs

Like Osaki et al. (98), five different PBDP were defined for CoNS episodes, which are the following:

- Pattern 1: one positive bottle out of four in two sets.
- Pattern 2: two positive bottles in one set (the other set remaining negative).
- Pattern 3: one positive bottle in each of two sets.
- Pattern 4: three positive bottles in two sets.
- Pattern 5: at least four positive bottles in two sets.

For the analysis of the TTP and the PBDP, paediatric patients were excluded because in this type of patient only a single vial is usually drawn.

Statistical analysis

The data are presented as mean and median TTP and IQR and analysed using the Mann–Whitney U, Wilcoxon W, and Wilcoxon signed-rank test using the SPSS V. 26 and the Epidat V. 4.2 programmes.

BLR analysis was used to examine the influence of TTP and PBDP in BCC by CoNS and to achieve a predicting formula for the probability of contamination based on these variables.

Objective 4: evaluate the impact of the SARS-CoV-2 pandemic on BC utilization and describe the characteristics of bacteraemia in SARS-CoV-2 patients.

Study design

Of all the episodes (real and contaminations) recorded prospectively for Objective 3, from February 28, 2020, to December 31, 2020, it was also recorded if the patient had SARS-CoV-2 confirmed by reverse transcription PCR (RT-PCR).

Laboratory methods and sample processing

Previously discussed in the analytical phase section.

Statistical analysis

Descriptive analysis and comparison of proportions was performed using the Chi square test, considering a 95% CI.

RESULTS

OBJECTIVE 1

Bloodstream infections: incidence and trends at a tertiary and a district hospital during a six-year period (2015-2020)

Summary

The aim of this retrospective study was to evaluate trends in workload, incidence, aetiology, and resistance profiles to key antibiotics in BSI over a six-year period (from 2015 to 2020) in a tertiary and a district hospital. A total of 96157 BCs was investigated, and 5426 BSI episodes were identified.

The number of BCs/1000 admissions requested remained stable in both hospitals. In HUA, BSI/1000 admissions incidence evolved from 18.0 episodes (2016) to 22.7/1000 (2020) admissions showing an annual increase of 0.77 episodes/1000 admissions (95% CI, 0.12-1.42; *P*=0.031). BSI/1000 admissions belonging to BCs ordered in the ED significantly increased in HUA, annual increase of 0.56 episodes/1000 admissions (95% CI, 0.18-0.93; *P*=0.015). These trends were not observed in HAD. The incidence of BSI caused by ESBL producing *Enterobacterales* and by MDR *P. aeruginosa* did not increase over the years, with rates ranging from 0.7 to 1.5 episodes/1000 admissions, and from 0.02 to 0.2 episodes/1000 admissions, respectively. The methicillin-resistant *S. aureus* (MRSA) rate stayed virtually unchanged. Resistance to glycopeptides and linezolid remained rare among *E. faecalis* and *E. faecium* strains.

Trends in BCs and BSI workload

The evolution of BC workload in both hospitals is presented in Table 8. Between 2015 and 2020, the Microbiology Laboratory investigated 96157 BCs, an average of 16026 per year (annual range 14088-16611).

The absolute number of BCs collected per year, as well as the incidence of BC/1000 admissions for both HUA and HAD, did not significantly fluctuate during the study period.

The number of BCs investigated per month is depicted in Figure 1. There is a marked peak in the number of BCs requested in March 2020, coinciding with the outbreak of the SARS-CoV-2 pandemic.

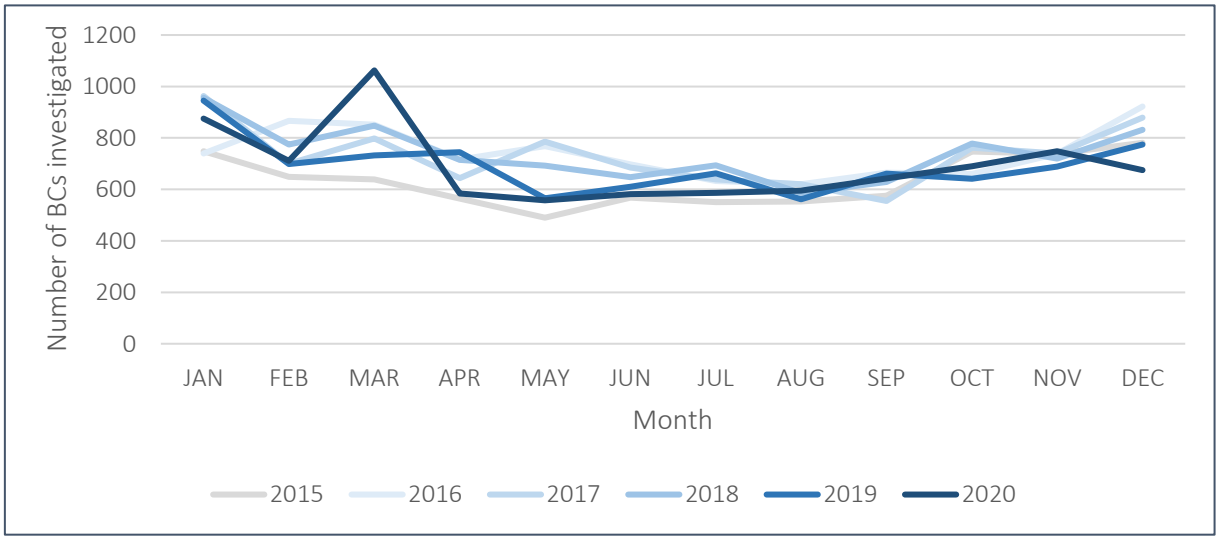


Figure 1. Number of BCs investigated per month. *BC: blood culture.*

In total, 5426 BSI episodes were identified. Concerning HUA, in adults, BSI incidence evolved from 17.9 episodes (2016) to 22.6/1000 admissions in 2020, showing an annual

increase of 0.82 episodes/1000 admissions (95% CI, 0.21-1.43; *P*=0.020); in children the incidence remained stable. In HAD, no upward trend was observed.

5.4% of the BSI episodes were polymicrobial (5.7% in HUA and 3.8% in HAD). The incidence of polymicrobial episodes significantly increased in HUA. No polymicrobial episodes were detected in children.

Table 8. Evolution of BCs ordered and of BSI episodes per 1000 admissions in the HUA and the HAD.

HOSPITAL			2015	2016	2017	2018	2019	2020	ANNUAL INCREASE RATIO	TREND DIRECTION	TREND (*P*-VALUE)
Total		BC	14088	16401	16252	16533	16272	16611	NS	NS	0.124
		BSI	822	877	917	917	949	944	23.60	↑	**0.008**
HUA	Total	BC/1000 adm.	326.8	331.2	337	338.6	338.3	389.1	NS	NS	0.065
		BSI/1000 adm.	18.4	18.0	19.1	18.9	19.9	22.7	0.77	↑	**0.031**
		BSI ED/1000 adm.	10.8	10.3	11.5	11.3	12.3	13.5	0.55	↑	**0.014**
		Polymicrobial BSI/1000 adm.	1.0	1.0	1.1	1.1	1.1	1.4	0.07	↑	**0.036**
		Mean age	67	68	69	69	68	69	NS	NS	0.157
		% Men	60.6	62.7	62.2	64.7	59.3	67.4	NS	NS	0.331
	Children (≤ 14 years)	BC/1000 adm.	778.2	889.7	1071.4	975.9	761.1	833.0	NS	NS	0.862
		BSI/1000 adm.	36.3	23.7	31.3	19.1	21.5	23.3	NS	NS	0.134
		BSI ED/1000 adm.	20.2	5.6	11.9	8.9	14.3	7.2	NS	NS	0.410
		Mean age	2	1	2	2	2	2	NS	NS	0.438
		% Men	44.4	70.6	66.7	73.3	73.3	76.9	NS	NS	0.056
	Adults (> 15 years)	BC/1000 adm.	318.3	321.1	324.7	326.5	331.1	382.2	NS	NS	0.066
		BSI/1000 adm.	18.0	17.9	18.9	18.9	19.8	22.6	0.82	↑	**0.020**
		BSI ED/1000 adm.	10.7	10.4	11.5	11.4	12.3	13.6	0.57	↑	**0.008**
		Mean age	70	69	71	71	70	70	NS	NS	0.684
		% Men	61.2	62.5	62	64.6	59.1	67.3	NS	NS	0.395
HAD		BC/1000 adm.	174.4	552.7	451.7	408.0	410.9	517.5	NS	NS	0.312
		BSI/1000 adm.	14.8	27.6	25.0	21.5	23.0	25.2	NS	NS	0.411
		Polymicrobial BSI/1000 adm.	0.2	0.9	0.7	1.3	1.5	0.6	NS	NS	0.317
		BSI ED/1000 adm.	6.9	23.8	22.3	18.3	20.5	22.6	NS	NS	0.256
		Mean age	56	67	70	68	69	69	NS	NS	0.119
		% Men	64.8	56.5	58.6	57.5	66.4	54.2	NS	NS	0.606

In bold, significant trend (*P*<0.05). *HUA: University Hospital of Araba, HAD: Hospital of Alto Deba, BC: blood culture, BSI: bloodstream infection, ED: Emergency Department, adm.: admissions, NS: not significant.*

Aetiology

As mentioned in the previous section, the incidence of bacteraemia increased from 2015 to 2020. The aetiology of the same was studied globally, year after year, per 1000 admissions.

Overall, in 54.1% of the episodes Gram-negative microorganisms were involved, 39.4% Gram positive bacteria, 4.5% strict anaerobes, and 2.0% yeasts.

The polymicrobial bacteraemia rate was significantly higher (*P*=0.028) in the tertiary hospital than in the district hospital.

The evolution of the aetiology of BSI episodes for HUA and HAD is depicted in Figure 2.

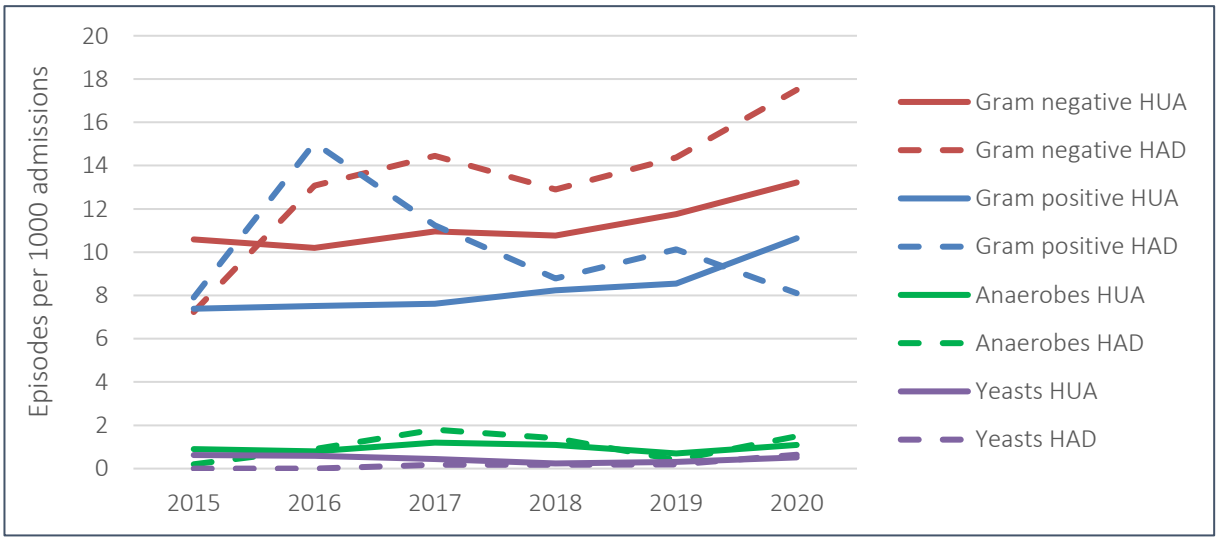


Figure 2. BSI episodes per 1000 admissions by type of microorganism in the HUA and in the HAD. *HUA: University Hospital of Araba, HAD: Hospital of Alto Deba.*

Table 9 summarizes the evolution of BSI per 1000 admissions by type of microorganism involved and hospital.

Table 9. Evolution of BSIs per 1000 admissions by type of microorganism involved and institution.

MICROORGANISM	HOSPITAL	2015	2016	2017	2018	2019	2020	ANNUAL INCREASE RATIO	TREND DIRECTION	TREND (*P*-VALUE)
Gram negative	HUA	10.6	10.2	11.0	10.8	11.8	13.2	0.50	↑	**0.026**
	HAD	7.2	13.1	14.4	12.9	14.4	17.5	1.54	↑	**0.032**
Gram positive	HUA	7.4	7.5	7.6	8.2	8.6	10.6	0.57	↑	**0.021**
	HAD	7.9	15.0	11.2	8.8	10.1	8.1	NS	NS	0.534
Anaerobes	HUA	0.9	0.8	1.2	1.1	0.7	1.1	NS	NS	0.739
	HAD	0.2	0.9	1.8	1.4	0.4	1.5	NS	NS	0.453
Yeasts	HUA	0.6	0.6	0.4	0.2	0.3	0.5	NS	NS	0.277
	HAD	0	0	0.2	0.2	0.2	0.6	0.10	↑	**0.022**

In bold, significant trend (P<0.05). *HUA: University Hospital of Araba, HAD: Hospital of Alto Deba, NS: not significant.*

In the HUA, a significant increase in BSI episodes per 1000 admissions caused by Gram-negative and Gram-positive bacteria was found. In the HAD, on the other hand, a significant increase in BSI episodes/1000 admissions causes by Gram-negative bacteria and yeasts was detected.

Excluding strict anaerobes, Gram-negative bacteria were predominant over Gram-positive ones. In total there were 3131 Gram-negative bacteria and 2283 Gram-positive bacteria involved.

The twelve most frequent causative agents of BSI by age range are displayed in Table 10.

Overall, *E. coli* was the most frequently involved microorganism. By age groups, *E. coli* was the most frequently isolated microorganism in adults (both in the 15 to 65 years

range, as well as in those over 65 years); in children the most frequently isolated microorganism was *S. epidermidis*.

The ESKAPE group (*E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa* and *Enterobacte*r spp.) accounted for 23.8% and 17.3% of the episodes in HUA and HAD, respectively, with incidences of 4.6 and 4.0 episodes/1000 admissions; no trend was observed.

K. pneumoniae was the only ESKAPEEc (ESKAPE group and *E. coli*) microorganism that significantly increased throughout the five years, annual increase of 0.09 episodes/1000 admissions (95% CI, 0.02-0.17; *P*=0.036); this trend was only observed in HUA.

Table 10. Age distribution of the top 12 pathogens isolated from BC specimens from 2015 to 2020.

	TOTAL ▪ Number of episodes: 5426 ▪ Monomicrobial episodes: 94.4% (5130) ▪ MA: 68 years ▪ Sex: 62.3% M ▪ ED: 62.9% (3414/5426)			CHILDREN (≤ 14 YEARS) ▪ Number of episodes: 152 ▪ Monomicrobial episodes: 100% (152) ▪ MA: 2 years (75.0% ≤1 years) ▪ Sex: 61.2% M ▪ ED: 35.5% (54/152)			ADULTS 15-64 YEARS ▪ Number of episodes: 1643 ▪ Monomicrobial episodes: 95.1% (1562) ▪ MA: 51 years ▪ Sex: 62.5 % M ▪ ED: 60.4% (992/1643)			ADULTS ≥ 65 YEARS ▪ Number of episodes: 3631 ▪ Monomicrobial episodes: 94.3% (3423) ▪ MA: 79 years ▪ Sex: 62.4% M ▪ ED: 65.1% (2365/3631)		
	n	Percentage	Rank	n	Percentage	Rank	n	Percentage	Rank	n	Percentage	Rank
Gram negative												
E. coli	2006	37.0	1	20	13.2	2	533	32.4	1	1453	40.0	1
K. pneumoniae	278	5.1	4	5	3.3	6	79	4.8	5	194	5.3	4
P. aeruginosa	193	3.6	7	0	0		46	2.8	9	147	4.0	6
Enterobacter spp.	117	2.2	10	2	1.3	8	46	2.8	10	69	1.9	11
K. oxytoca	93	1.7	12	1	0.7	9	15	0.9	--	77	2.1	9
P. mirabilis	85	1.6	--	0	0	--	17	1.0	12	68	1.9	12
Bacteroides fragilis Group	114	2.1	11	0	0	0	48	2.9	8	66	1.8	--
Gram positive												
S. aureus	485	8.9	2	20	13.2	3	160	9.7	3	305	8.4	2
S. epidermidis	467	8.6	3	41	27.0	1	181	11.0	2	245	6.7	3
S. hominis	128	2.4	9	16	10.5	4	35	2.1	11	77	2.1	10
E. faecalis	216	4.0	6	2	1.3	9	61	3.7	6	153	4.2	5
E. faecium	167	3.1	8	2	1.3	10	49	3.0	7	116	3.2	8
S. pneumoniae	241	4.4	5	11	7.2	5	100	6.1	4	130	3.6	7
S. agalactiae	67	1.2	--	5	3.3	7	--	--	--	--	--	--

n: number, MA: mean age, M: male, ED: Emergency Department.

Gram-negative microorganisms

Gram-negative bacteria were the most frequent cause of BSI in both institutions with annual averages of 11.2 and 13.3 episodes/1000 admissions in HUA and HAD.

Their incidences significantly increased throughout the study period, evolving from 10.2 (2016) to 13.2 (2020) episodes/1000 admissions in HUA, annual increase of 0.50 episodes/1000 admissions (95% CI, 0.10-0.90; *P*=0.026), and from 7.2 (2015) to 17.5 (2020) episodes per 1000 admissions in HAD, annual increase of 1.54 episodes/1000 admissions (95% CI, 0.21-2.87; *P*=0.032). The evolution of BSI episodes caused by Gram-negative microorganisms is presented in Table 11.

The incidence per 1000 admissions of *Enterobacterales* significantly increased in HAD at an annual rate of 1.54 episodes/1000 admissions (95% CI, 0.33-2.75; *P*=0.024). In HUA, the incidence rate remained more stable.

E. coli was recovered in 2006 episodes, annual mean rate of 7.0 and 9.7 episodes/1000 admissions in both HUA and HAD. The incidence increased progressively from 5.9 (2015) to 12.2 (2020) episodes/1000 admissions in HAD, although this increase was not statistically significant. In HUA, on the other hand, the incidence rate remained more stable.

As mentioned before, the recovery of *K. pneumoniae* significantly increased throughout the five years, annual increase of 0.09 episodes/1000 admissions (95% CI, 0.02-0.17; *P*=0.036); this trend was only observed in HUA.

The mean annual incidence per 1000 admissions of *Enterobacter* spp. was 0.5 episodes per 1000 admissions in HUA, and 0.3 in HAD. For *Proteus* spp. mean annual incidences were 0.3 episodes/1000 admissions in both institutions. For *Serratia* spp. and *Citrobacter* spp. mean annual incidences per 1000 admissions were 0.2 in both hospitals. *Salmonella* spp. annual mean incidences remained low in HUA and HAD (< 0.1 episodes/1000 admissions).

NFGNB were responsible of 4.6% of the BSIs, with annual mean incidences/1000 admissions of 0.9 (HUA) and 0.7 (HAD).

For *P. aeruginosa*, specifically, annual mean incidences per 1000 admissions were 0.7 (HUA) and 0.6 (HAD).

Only three episodes of bacteraemia caused by *A. baumannii* were recovered; all three episodes occurred in HUA in 2017. On the other hand, the incidence of *Stenotrophomonas maltophilia* remained low in HUA (< 0.1 episodes / 1000 admissions) and no case was isolated in HAD.

No trend in BSI caused by neither NFGNB nor by *P. aeruginosa*, specifically, was observed.

Invasive *Haemophilus* spp. was involved in 24 episodes (0.4%) and *H. influenzae* was the most frequently isolated species (20/24; 83.3%); of these, 75.0% of the episodes occurred in adult patients.

During the study period there were two episodes of BSI due to *N. gonorrhoeae*, one in 2019 and one in 2020. These cases will be discussed in Appendix I.

Table 11. Gram negative bacteria: evolution of BSIs per 1000 admissions by microorganism involved and institution.

MICROORGANISM	HOSPITAL	2015	2016	2017	2018	2019	2020	ANNUAL INCREASE RATIO	TREND DIRECTION	TREND (*P*-VALUE)
Gram negative bacteria	HUA	10.6	10.2	11.0	10.8	11.8	13.2	0.50	↑	**0.026**
	HAD	7.2	13.1	14.4	12.9	14.4	17.5	1.54	↑	**0.032**
Enterobacterales	HUA	9.9	9.1	9.7	9.7	10.4	11.5	NS	NS	0.072
	HAD	6.4	12.0	12.5	11.5	13.3	16.6	1.54	↑	**0.024**
ESBL	HUA	0.9	0.8	0.9	1.0	0.8	0.9	NS	NS	0.725
	HAD	0.7	1.1	0.7	1.3	0.9	1.5	NS	NS	0.116
E. coli	HUA	7.3	6.2	7.3	6.9	7.0	7.3	NS	NS	0.634
	HAD	5.9	9.9	10.3	8.4	12.0	12.2	NS	NS	0.050
ESBL	HUA	0.8	0.7	0.9	0.9	0.7	0.7	NS	NS	0.394
	HAD	0.7	1.1	0.7	0.7	0.9	1.1	NS	NS	0.374
Klebsiella spp.	HUA	1.3	1.2	1.4	1.5	1.7	2.1	0.16	↑	**0.010**
	HAD	0.7	1.4	1.8	2.5	1.3	2.8	NS	NS	0.090
Proteus spp.	HUA	0.3	0.3	0.3	0.5	0.3	0.4	NS	NS	0.374
	HAD	0	0.4	0.2	0	0.4	0.9	NS	NS	0.139
Enterobacter spp.	HUA	0.4	0.6	0.2	0.4	0.4	0.8	NS	NS	0.405
	HAD	0.2	0.4	0.2	0.2	0.6	0.4	NS	NS	0.277
Citrobacter spp.	HUA	0.2	0.1	0.1	0.1	0.1	0.3	NS	NS	0.482
	HAD	0	0.2	0	0.5	0.2	0	NS	NS	0.800
Serratia spp.	HUA	0.3	0.2	0.1	0.1	0.2	0.2	NS	NS	0.435
	HAD	0	0.2	0.2	0.4	0	0.4	NS	NS	0.261
Salmonella spp.	HUA	0	0.1	0	0	0.1	0.1	NS	NS	0.595
	HAD	0	0.2	0.2	0	0	0	NS	NS	0.428
NFGNB	HUA	0.6	1.0	1.0	0.8	1.0	1.3	NS	NS	0.079
	HAD	0	0.4	1.4	0.9	0.7	0.6	NS	NS	0.448
P. aeruginosa	HUA	0.5	0.8	0.8	0.6	0.7	1.0	NS	NS	0.195
	HAD	0	0.4	1.1	0.7	0.6	0.6	NS	NS	0.344
S. maltophilia	HUA	0.1	0	0	0	0.1	0.1	NS	NS	0.643

In bold, significant trend ($P<0.05$). *HUA: University Hospital of Araba, HAD: Hospital of Alto Deba, NS: not significant, ESBL: extended-spectrum beta-lactamase, NFGNB: non-fermenting Gram-negative bacilli.*

Gram-positive microorganisms

Gram-positive bacteria participated in 2283 episodes, mean annual incidence of 8.3 and 10.2 episodes/1000 admissions in HUA and HAD, respectively. In HUA, the incidence statistically increased throughout the study period at an annual rate of 0.57 episodes/1000 admissions (95% CI, 0.14-1.00; *P*=0.021); in HAD no such trend was observed.

The evolution of BSI episodes caused by Gram-positive microorganisms is summarized in Table 12.

S. aureus caused 485 episodes of BSI, with a mean annual incidence of 1.8 episodes/1000 admissions in HUA, and 1.6 in HAD, no trend was noted in neither of the two hospitals.

The mean annual incidence of CoNS BSI episodes increased significantly in HUA at an annual rate of 1.6 episodes/1000 admissions (95% CI, 0.02-0.30; *P*=0.036), while it decreased, also significantly, in HAD.

No significant increase in the rate of BSI caused by enterococci was observed, and mean annual incidences were 1.6 and 0.8 episodes/1000 admissions in HUA and HAD. *E. faecalis* was more frequent than *E. faecium*, being involved in 216 total episodes versus 167.

Interestingly, the incidence of BSI caused by *S. pneumoniae* significantly increased in HUA from 2015 to 2019 at an annual rate of 0.1 episodes/1000 admissions (95% CI, 0.09-0.15; *P*=0.001), but the recovery rate drastically decreased in 2.5% (95% CI, 0.69-4.31; *P*=0.007)

and 8.7% (95% CI, 2.5-14.9; *P*=0.008) in HUA and HAD, respectively, coinciding with the advent of the SARS-CoV-2 pandemic.

Eleven episodes of *S. pneumoniae* BSI occurred in children, one of them in a newborn whose case will be discussed in Appendix II. The most frequent *S. pneumoniae* serotype was 8 (21.8%), followed by 3 (11.9%) and 12F (10.4%).

S. pyogenes was responsible of 34 BSI episodes, only one occurring in a child. The incidences per 1000 admissions were of 0.1 and 0.2 for HUA and HAD, and no trend was observed. Only one episode occurred in a child (men age was 66 years), and 52.8% of the episodes occurred in men.

A total of 27 episodes caused by *L. monocytogenes* were isolated. The incidence per 1000 admissions was 0.1 in HUA and 0.2 in the HAD, without fluctuations over the years. Mean age was 73 years, and 55.6% of the episodes occurred in women.

Table 12. Gram positive bacteria: evolution of BSIs per 1000 admissions by microorganism involved and institution.

MICROORGANISM	HOSPITAL	2015	2016	2017	2018	2019	2020	ANNUAL INCREASE RATIO	TREND DIRECTION	TREND (*P*-VALUE)
Gram positive bacteria	HUA	7.4	7.5	7.6	8.2	8.6	10.6	0.57	↑	**0.021**
	HAD	7.9	15.0	11.2	8.8	10.1	8.1	NS	NS	0.534
CoNS	HUA	2.2	2.1	2.1	2.3	2.8	2.9	0.16	↑	**0.036**
	HAD	2.7	3.4	1.6	1.8	1.5	0.9	-0.42	↓	**0.028**
S. aureus	HUA	1.8	1.7	1.7	1.5	1.9	2.2	NS	NS	0.275
	HAD	1.5	3.0	2.3	1.1	0.6	1.3	NS	NS	0.231
MRSA	HUA	0.4	0.3	0.4	0.3	0.4	0.3	NS	NS	0.643
	HAD	0	0.2	0.4	0.4	0	0.4	NS	NS	0.455
S. pneumoniae	HUA	0.5	0.6	0.8	0.8	1.0	0.5	NS	NS	0.523
	HAD	0.8	2.9	3.4	2.2	2.6	0.6	NS	NS	0.784
Enterococcus spp.	HUA	1.4	1.6	1.6	1.6	1.3	2.0	NS	NS	0.349
	HAD	0.3	1.1	0.2	0.7	1.5	0.9	NS	NS	0.303
S. pyogenes	HUA	0.1	0	0.1	0.2	0.2	0.1	NS	NS	0.302
	HAD	0	0	0.2	0.4	0.2	0.2	NS	NS	0.140
L. monocytogenes	HUA	0.1	0.1	0.1	0.1	0	0.1	NS	NS	0.374
	HAD	0	0.5	0.2	0.2	0	0.2	NS	NS	0.783

In bold, significant trend ($P<0.05$). *HUA: University Hospital of Araba, HAD: Hospital of Alto Deba, NS: not significant, CoNS: coagulase-negative staphylococci, MRSA: methicillin-resistant Staphylococcus aureus.*

Anaerobic bacteria

Overall, strict anaerobic microorganisms were involved in 263 episodes (4.8%), annual mean rate of 1.0 episodes/1000 admissions in both institutions; 21.7% were involved in polymicrobial BSI. No trend in the incidence of anaerobic BSI was observed.

Mean age of the patients with anaerobic bacteraemia was 68 years. No anaerobic BSI was detected in children.

Anaerobic microorganisms most isolated included those belonging to the *Bacteroides fragilis* Group (BFG) with 114 episodes (43.3%) and a mean annual incidence of 0.4 and 0.5 episodes/1000 admissions in HUA and HAD, respectively and *Clostridium* spp., with 65 episodes (24.7%) and a mean annual rate of 0.2 episodes per 1000 admissions for both institutions (Table 13). The distribution of anaerobic species is depicted in Figure 3.

Table 13. Strict anaerobes: evolution of BSIs per 1000 admissions by microorganism involved and institution.

MICROORGANISM	HOSPITAL	2015	2016	2017	2018	2019	2020	ANNUAL INCREASE RATIO	TREND DIRECTION	TREND (*P*-VALUE)
Strict Anaerobes	HUA	0.9	0.8	1.2	1.1	0.7	1.1	NS	NS	0.739
	HAD	0.2	0.9	1.8	1.4	0.4	1.5	NS	NS	0.453
Gram-negative anaerobes	HUA	0.7	0.5	0.7	0.6	0.4	0.5	NS	NS	0.186
	HAD	0.2	0.7	0.9	0.7	0.4	1.3	NS	NS	0.201
BFG	HUA	0.6	0.4	0.5	0.3	0.3	0.4	NS	NS	0.137
	HAD	0	0.5	0.7	0.4	0.2	1.1	NS	NS	0.217
Gram-positive anaerobes	HUA	0.2	0.3	0.4	0.5	0.3	0.6	NS	NS	0.075
	HAD	0	0.2	0.9	0.7	0	0.2	NS	NS	0.941
Clostridium spp.	HUA	0.2	0.3	0.4	0.2	0.2	0.2	NS	NS	0.519
	HAD	0	0.2	0.4	0.4	0	0.2	NS	NS	0.734

HUA: University Hospital of Araba, HAD: Hospital of Alto Deba, NS: not significant, BFG: Bacteroides fragilis Group.

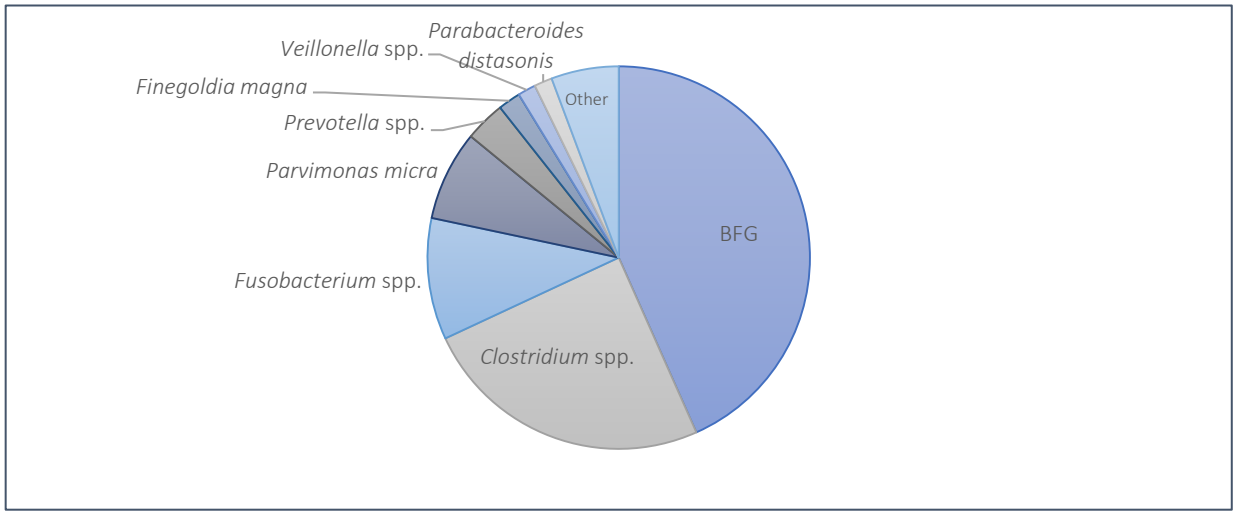


Figure 3. Species distribution in anaerobic BSI. *BFG: Bacteroides fragilis Group.*

Yeasts

Yeasts were recovered in 115 episodes (2.1%), one episode belonging to an infant. Mean age of the patients with fungaemia was 68 years.

Fungal BSI presented an annual mean rate of 0.5 and 0.2 episodes/1000 admissions in HUA and HAD. A significant increase in the incidence was observed in HAD (annual increase of 0.10 episodes/1000 admissions (95% CI, 0.03-0.18; *P*=0.022). The evolution of BSI episodes caused by *Candida* spp. is displayed in Table 14.

C. albicans, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei* accounted for 92.9% of candidaemia.

C. albicans was the most frequently isolated yeast (42.6%), followed by *C. glabrata* (25.2%) and *C. parapsilosis* (18.3%). Non-albicans *Candida* spp. accounted for 55.7% of

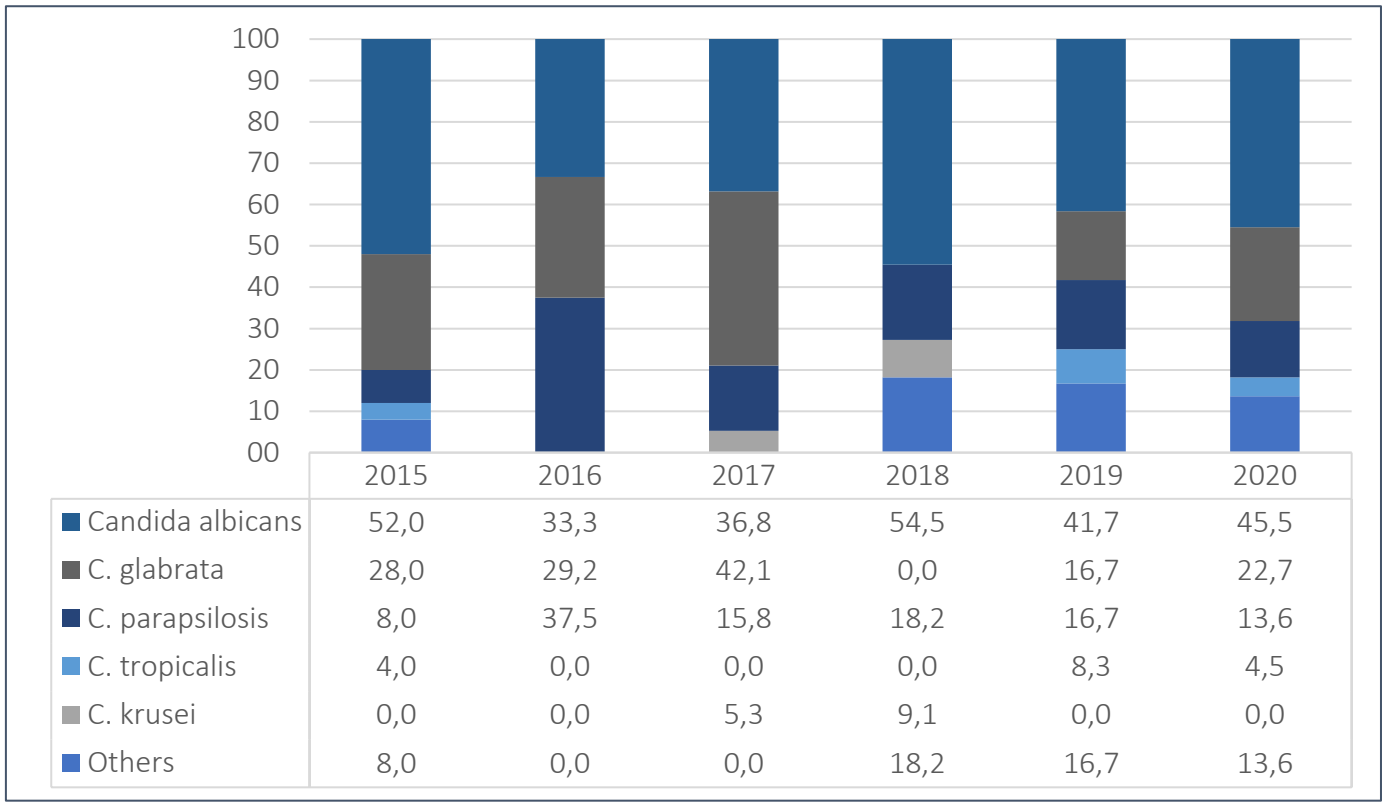
the fungal BSI (Figure 4) and a significant increase in the incidence of BSI by this group was observed in HAD (annual increase of 0.07 episodes/1000 admissions (95% CI, 0.03-0.12; *P*=0.012) but not in HUA.

Cryptococcus neoformans was responsible of two BSI episodes diagnosed in 2019 in two HIV patients.

Table 14. Yeasts: evolution of BSIs per 1000 admissions by microorganism involved and institution.

MICROORGANISM		HOSPITAL	2015	2016	2017	2018	2019	2020	ANNUAL INCREASE RATIO	TREND DIRECTION	TREND (*P*-VALUE)
Yeasts		HUA	0.6	0.6	0.4	0.2	0.3	0.5	NS	NS	0.277
		HAD	0	0	0.2	0.2	0.2	0.6	0.10	↑	**0.022**
	C. albicans	HUA	0.3	0.2	0.2	0.1	0.1	0.2	NS	NS	0.158
		HAD	0	0	0	0	0	0.2	NS	NS	0.170
	Non-albicans *Candida* spp.	HUA	0.3	0.4	0.3	0.1	0.1	0.3	NS	NS	0.309
		HAD	0	0	0.2	0.2	0.2	0.4	0.07	↑	**0.012**

In bold, significant trend (*P*<0.05). *HUA: University Hospital of Araba, HAD: Hospital of Alto Deba, NS: not significant.*



	2015	2016	2017	2018	2019	2020
Candida albicans	52,0	33,3	36,8	54,5	41,7	45,5
C. glabrata	28,0	29,2	42,1	0,0	16,7	22,7
C. parapsilosis	8,0	37,5	15,8	18,2	16,7	13,6
C. tropicalis	4,0	0,0	0,0	0,0	8,3	4,5
C. krusei	0,0	0,0	5,3	9,1	0,0	0,0
Others	8,0	0,0	0,0	18,2	16,7	13,6

Figure 4. Species distribution of *Candida* spp. *Others comprises the following species: C. dubliniensis*, *C. famata*, *C. guilliermondii*, *C. kefyr* and *C. metapsilosis*.

BSI in the ED

Table 15 summarizes the evolution of BSIs belonging to BCs drawn in both EDs.

A total of 3414 BSI episodes (62.9%) belonged to BCs ordered in the ED, 59.7% in HUA, and 81.0% in HAD.

The incidence of BSI/1000 admissions belonging BCs drawn in the ED of HUA increased significantly with an annual increase rate of 0.56 episodes/1000 admissions (95% CI, 0.18-0.93; *P*=0.015), while this trend was not observed in HAD.

In HUA, mean age was 70 years, 60.5% of the episodes occurred in men and 6.0% of the BSI were polymicrobial; in HAD mean age was 71 years, 57.2% of the episodes occurred in men and 3.9% were polymicrobial.

In HUA, *E. coli* was the most frequently recovered microorganism, followed by *S. aureus* and *S. pneumoniae*; in HAD, however, *E. coli* was followed by *S. pneumoniae* and *K. pneumoniae*.

Of the twelve microorganisms most frequently recovered from BC drawn in the ED of the HUA, *P. mirabilis* was the only one whose incidence increased significantly, with an annual increase rate of 0.1 episodes/1000 admissions (95% CI, 0.01-0.11; *P*=0.037)

The incidence of ESKAPE pathogens recovered from BCs ordered in the ED was 2.1 (HUA) and 3.0 (HAD) episodes/1000 admissions, with no trend over the years observed.

Table 15 A. Evolution of BSIs per 1000 admissions of BC performed in the ED of HUA.

HUA		2015	2016	2017	2018	2019	2020	ANNUAL INCREASE RATIO	TREND (*P*-VALUE)
Total		10.8	10.3	11.5	11.3	12.3	13.5	0.56	**0.015**
ESKAPE		1.9	2.0	2.0	1.6	2.4	2.9	NS	0.153
Rank									
1	*E. coli*	5.7	4.8	5.6	5.3	5.7	6.0	NS	0.353
	ESBL	0.6	0.4	0.7	0.7	0.6	0.5	NS	1
2	*S. aureus*	0.7	0.7	0.9	0.6	0.8	0.9	NS	0.43
	MRSA	0.2	0.1	0.2	0.1	0.1	0.1	NS	0.116
3	*S. pneumoniae*	0.4	0.5	0.7	0.8	0.8	0.5	NS	0.261
4	*K. pneumoniae*	0.6	0.5	0.6	0.4	0.7	0.8	NS	0.316
5	*E. faecalis*	0.3	0.5	0.3	0.5	0.3	0.5	NS	0.714
6	*P. aeruginosa*	0.3	0.5	0.3	0.2	0.5	0.6	NS	0.357
7	*S. epidermidis*	0.4	0.3	0.3	0.2	0.6	0.2	NS	1
8	*Enterobacter* spp.	0.2	0.3	0.1	0.2	0.2	0.5	NS	0.284
9	*S. hominis*	0.4	0.2	0.2	0.3	0.1	0.2	NS	0.201
10	*P. mirabilis*	0.1	0.1	0.1	0.3	0.2	0.4	0.06	**0.037**
11	*S. agalactiae*	0.1	0.2	0.2	0.2	0.1	0.3	NS	0.158
12	BFG	0.2	0.2	0.3	0.1	0.1	0.2	NS	0.435

In bold, significant trend ($P<0.05$). *NS: not significant, ESKAPE: Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, P. aeruginosa and Enterobacter spp., ESBL: extended-spectrum beta-lactamase, MRSA: methicillin-resistant Staphylococcus aureus, BFG: Bacteroides fragilis Group.*

Table 15 B. Evolution of BSIs per 1000 admissions of BC performed in the ED of HAD.

HAD		2015	2016	2017	2018	2019	2020	ANNUAL INCREASE RATIO	TREND (*P*-VALUE)
Total		6.9	23.8	22.3	18.3	20.5	22.6	NS	0.258
ESKAPE		0.8	3.4	4.5	3.2	1.8	4.3	NS	0.402
Rank									
1	*E. coli*	2.9	8.2	9.5	7.3	9.8	10.7	NS	0.060
	ESBL	0.5	0.9	0.7	0.5	0.9	0.9	NS	0.319
2	*S. pneumoniae*	0.7	2.9	3.2	1.8	2.2	0.6	NS	0.743
3	*K. pneumoniae*	0.2	0.7	1.4	1.6	0.7	2.3	NS	0.077
4	*S. aureus*	0.3	2.1	1.6	0.5	0.2	0.6	NS	0.469
	MRSA	0	0.2	0.4	0.2	0	0.2	NS	0.777
5	*P. aeruginosa*	0	0.4	1.1	0.7	0.6	0.6	NS	0.303
6	*S. epidermidis*	0.5	0.7	0.2	0.9	0.6	0.4	NS	0.914
7	BFG	0	0.5	0.7	0.4	0.2	1.1	NS	0.266
8	*S. hominis*	0	0.9	0.7	0	0.6	0.4	NS	0.900
9	*E. faecalis*	0	0.9	0	0.4	0.9	0.2	NS	0.708
10	*S. agalactiae*	0.5	0.4	0	0.4	0.2	0.6	NS	0.814
11	*P. mirabilis*	0	0.4	0.2	0	0.4	0.9	NS	0.127
12	*Enterobacter* spp.	0.2	0.2	0.2	0.2	0.2	0.4	NS	0.138

NS: not significant, ESKAPE: Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, P. aeruginosa and Enterobacter spp., ESBL: extended-spectrum beta-lactamase, MRSA: methicillin-resistant Staphylococcus aureus, BFG: Bacteroides fragilis Group.

Antimicrobial resistance patterns

Gram-negative microorganisms

The ESBL phenotype was observed in 8.7% (244/2801) of the *Enterobacterales* and the incidence did not fluctuate over the years. No episode caused by an ESBL-producing *Enterobacterales* was obtained in children. Likewise, 0.7% (21/2801) of the *Enterobacterales* yielded elevated ertapenem MICs (> 0.5 mg/L), but carbapenemase production could only be demonstrated in 0.2% (6/2801); no trend was observed.

The ESBL-phenotype was present in 10.8% of the *E. coli* strains; the annual mean incidence/1000 admissions was 0.9 and 1.0 in HUA and HAD, no significant increase was observed in neither of the two hospitals. The mean age of the patients with BSI due to ESBL producing *E. coli* was 77 years (76 in HUA and 82 in HAD) and the majority (61.6%) were men. None of the *E. coli* ESBL isolates was recovered from children. The piperacillin-tazobactam susceptibility rates for ESBL- producing *E. coli* ranged from 64.9% to 92.7%.

The AmpC phenotype was observed in eight *E. coli* strains (0.4%), all isolated in the last four years of study. The recovery of this type of strains statistically increased in 0.04 (95% CI, 0.02-0.06; *P*=0.007). The mean age of patients with BSI caused by AmpC *E. coli* was 88 years (all were adults) and all were recovered in HUA. All strains were susceptible to ertapenem ($\leq$ 0.5 mg/L) and all except one were susceptible to cefepime (MIC $\leq$ 4 mg/L).

Although the presence of carbapenemases was not demonstrated in any of the *E. coli* isolates, five ESBL producing strains harboured ertapenem MICs greater than 0.5 mg/L; nevertheless, all isolates were susceptible to imipenem (MIC $\leq$ 2 mg/L) (Table 16).

Table 16. Antimicrobial non-resistance rates of *E. coli*.

				PERCENT OF ANTIMICROBIAL NON-RESISTANCE								
		YEAR	TOTAL	AMC ≤ 8	PIT ≤ 8	CTA ≤ 2	CTZ ≤ 4	CEP ≤ 4	ERT ≤ 0.5	CIP ≤ 0.5	GEN ≤ 2	AMI ≤ 8
E. coli	Total (2006)	2015	327	79.2	92.7	88.7	90.8	90.2	100	76.1	80.7	98.8
		2016	303	79.9	96.0	87.8	88.1	89.8	100	70.6	86.5	99.0
		2017	357	84.0	94.1	87.7	91.6	92.7	99.2	72.8	88.2	98.9
		2018	338	76.6	95.0	86.4	89.6	91.4	99.4	67.8	86.7	99.7
		2019	357	80.4	93.3	90.8	93.6	92.7	99.4	77.9	89.1	98.6
		2020	324	82.4	96.0	89.5	91.7	92.6	99.4	78.1	86.4	98.1
		Total	2006	80.5	94.5	88.5	91.0	91.6	99.8	74.0	86.5	99.1
	None/others* (1782)	2015	290	84.1	96.2	99.3	99.3	100	100	83.4	85.5	99.7
		2016	269	83.3	96.7	98.5	98.1	100	100	78.1	89.6	100
		2017	315	85.1	94.9	99.0	98.7	100	100	80.6	91.4	99.4
		2018	296	78.0	95.6	98.3	98.6	100	100	75.3	89.9	100
		2019	322	84.5	95.7	100	100	100	100	84.8	92.5	100
		2020	290	87.2	97.9	100	100	99.7	100	85.2	90.3	99.3
		Total	1782	83.7	96.1	99.2	99.2	99.9	100	81.3	90	99.7
	ESBL (216)	2015	37	40.5	64.9	5.4	24.3	13.5	100	18.9	43.2	91.9
		2016	34	52.9	91.2	2.9	8.8	8.8	100	11.8	61.8	91.2
		2017	41	78.0	90.2	2.4	36.6	36.6	95.1	14.6	65.9	97.6
		2018	41	68.3	92.7	0	26.8	29.3	97.6	14.6	65.9	100
		2019	33	45.5	75.8	3.0	33.3	21.2	100	15.2	60.6	90.9
		2020	30	46.7	83.3	0	23.3	23.3	93.3	16.7	46.7	90.0
		Total	216	56.5	83.3	2.3	25.9	22.7	97.7	15.3	57.9	94.0

*Others: non-ESBL, -AmpC, or -carbapenemase producing strains. *ESBL: extended-spectrum beta-lactamase, AMC: amoxicillin-clavulanate, PIT: piperacillin-tazobactam, CTA: cefotaxime, CTZ: ceftazidime, CEP: cefepime, ERT: ertapenem, CIP: ciprofloxacin, GEN: gentamicin, AMI: amikacin.*

The antimicrobial resistance profiles of *E. coli* in adult and paediatric BSIs are depicted in Figure 5.

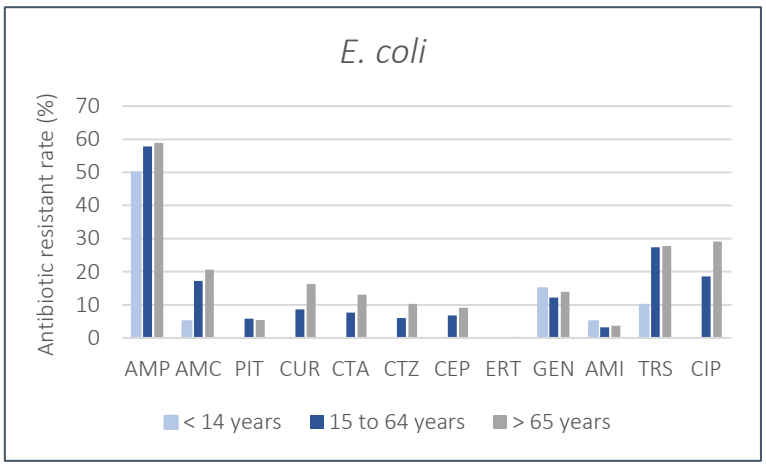


Figure 5. Antimicrobial resistance profiles of *E. coli* by age groups. *AMP: ampicillin, AMC: amoxicillin-clavulanate, PIT: piperacillin-tazobactam, CUR: cefuroxime, CTA: cefotaxime, CTZ: ceftazidime, CEP: cefepime, ERT: ertapenem, GEN: gentamicin, AMI: amikacin, TRS: trimethoprim-sulfamethoxazole, CIP: ciprofloxacin*.

For *K. pneumoniae*, amoxicillin-clavulanate susceptibility significantly decreased (*P*=0.018) throughout the study period.

The ESBL phenotype was observed in 6.1% (17/278) isolates, whereas the pAmpC was present only in two (0.7%). The annual mean incidence per 1000 admissions of ESBL-producing *K. pneumoniae* was 0.1 in both institutions, no significant trend was noted. This phenotype was observed in adult patients with a mean age of 69 years; the majority were men (84.6%).

Carbapenemase production could be demonstrated in four strains (1.4%). All the episodes in which a carbapenemase-producing strain of *K. pneumoniae* was involved occurred in adult men, mean age 65 years.

The antimicrobial non-resistance rates of *K. pneumoniae* to key antibiotics are presented in Table 17 and the antimicrobial resistance profiles by age-range are depicted in Figure 6.

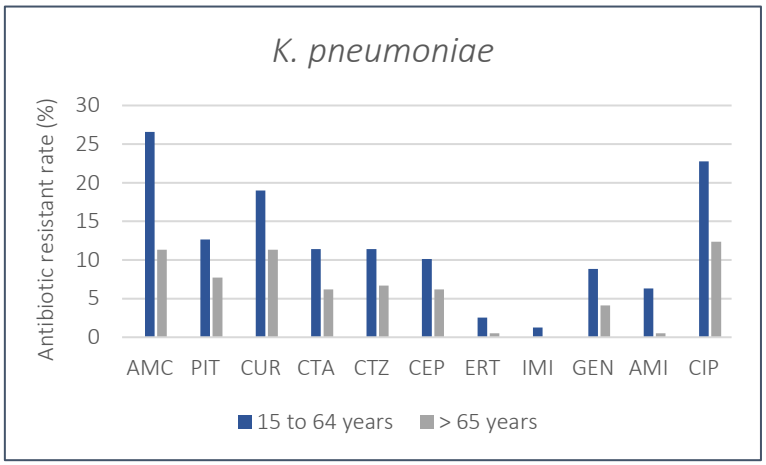


Figure 6. Antimicrobial resistance profiles of *K. pneumoniae* by age groups. *No strain resistant to the antibiotics presented belonging to paediatric BSI was recovered. AMC: amoxicillin-clavulanate, PIT: piperacillin-tazobactam, CUR: cefuroxime, CTA: cefotaxime, CTZ: ceftazidime, CEP: cefepime, ERT: ertapenem, IMI: imipenem, GEN: gentamicin, AMI: amikacin, CIP: ciprofloxacin*.

Table 17. Antimicrobial non-resistance rates of *K. pneumoniae.*

		PERCENT OF ANTIMICROBIAL NON-RESISTANCE									
		YEAR	TOTAL	AMC ≤ 8	PIT ≤ 8	CTA ≤ 2	CTZ ≤ 4	CEP ≤ 4	ERT ≤ 0.5	CIP ≤ 0.5	GEN ≤ 2	AMI ≤ 8
K. pneumoniae	Total (278)	2015	36	91.7	94.4	94.4	94.4	94.4	97.2	88.9	97.2	97.2
		2016	40	92.5	95.0	92.5	92.5	92.5	100	87.5	95.0	100
		2017	45	88.9	97.8	100	100	100	100	88.9	97.8	100
		2018	47	78.7	87.2	85.1	87.2	85.1	97.9	78.7	89.4	93.6
		2019	48	83.3	93.8	95.8	95.8	97.9	100	91.7	95.8	100
		2020	62	77.4	82.3	87.1	85.5	88.7	98.4	82.3	93.5	96.8
		Total	278	**84.5**	91.0	92.1	92.1	92.8	98.9	86.0	94.6	97.8
	None/others* (255)	2015	34	97.1	100	100	100	100	100	94.1	100	100
		2016	37	94.6	97.3	100	100	100	100	91.9	97.3	100
		2017	45	88.9	97.8	100	100	100	100	88.9	97.8	100
		2018	40	90.0	95.0	100	100	100	100	90.0	97.5	100
		2019	45	86.7	95.6	100	100	100	100	95.6	95.6	100
		2020	54	87.0	90.7	100	98.1	98.1	100	92.6	100	100
		Total	255	**90.2**	**95.7**	100	99.6	99.6	100	92.2	98.0	100
	ESBL (17)	2015	1	0	0	0	0	0	100	0	0	0
		2016	3	66.7	66.7	0	0	0	100	33.3	66.7	100
		2017	0	0	0	0	0	0	0	0	0	0
		2018	6	16.7	50.0	16.7	16.7	16.7	100	16.7	33.3	66.7
		2019	2	50.0	50.0	0	0	0	100	50.0	100	100
		2020	5	20.0	40.0	0	0	0	100	0	40.0	100
		Total	17	29.4	47.1	5.9	5.9	5.9	100	17.6	47.1	82.4

In bold, significant trend (P<0.05). *Others: non-ESBL, -AmpC, or -carbapenemase producing strains. *ESBL: extended-spectrum beta-lactamase, AMC: amoxicillin-clavulanate, PIT: piperacillin-tazobactam, CTA: cefotaxime, CTZ: ceftazidime, CEP: cefepime, ERT: ertapenem, CIP: ciprofloxacin, GEN: gentamicin, AMI: amikacin.*

For *Enterobacter* spp., cefepime and ertapenem MICs were ≤ 0.5 mg/L (susceptible) in 88.0% and 89.7% of the isolates, respectively, and the piperacillin-tazobactam susceptibility was 79.5% (Table 18).

Carbapenemase production could be demonstrated in only two strains, although elevated ertapenem MICs were observed in twelve. All isolates harboured imipenem MICs ≤ 4 mg/L. The two episodes in which a carbapenemase-producing strain of *Enterobacter* spp. was involved occurred in adult men, aged < 65 years (mean age 52 years).

The overall susceptibility rates to aminoglycosides and ciprofloxacin ranged from 77.8 to 100%.

Table 18. Antimicrobial non-resistance rates of *Enterobacter* spp.

	PERCENT OF ANTIMICROBIAL NON-RESISTANCE									
	YEAR	TOTAL	AMC ≤ 8	PIT ≤ 8	CTA ≤ 2	CTZ ≤ 4	CEP ≤ 4	ERT ≤ 0.5	CIP ≤ 0.5	GEN ≤ 2	AMI ≤ 8
Enterobacter spp.	2015	17	NA	82.4	94.1	94.1	94.1	94.1	82.4	88.2	100
	2016	27	NA	77.8	74.1	77.8	85.2	85.2	85.2	92.6	96.3
	2017	9	NA	77.8	66.7	88.9	100	88.9	88.9	100	100
	2018	16	NA	81.3	75.0	75.0	100	93.8	93.8	93.8	100
	2019	18	NA	77.8	66.7	72.2	88.9	88.9	77.8	88.9	100
	2020	30	NA	80.0	70.0	73.3	90.0	90.0	80.0	86.7	100
	Total	117	NA	79.5	74.4	78.6	91.5	89.7	83.8	90.6	99.1

AMC: amoxicillin-clavulanate, PIT: piperacillin-tazobactam, CTA: cefotaxime, CTZ: ceftazidime, CEP: cefepime, ERT: ertapenem, CIP: ciprofloxacin, GEN: gentamicin, AMI: amikacin.

The antimicrobial resistance profiles by age range are shown in Figure 7.

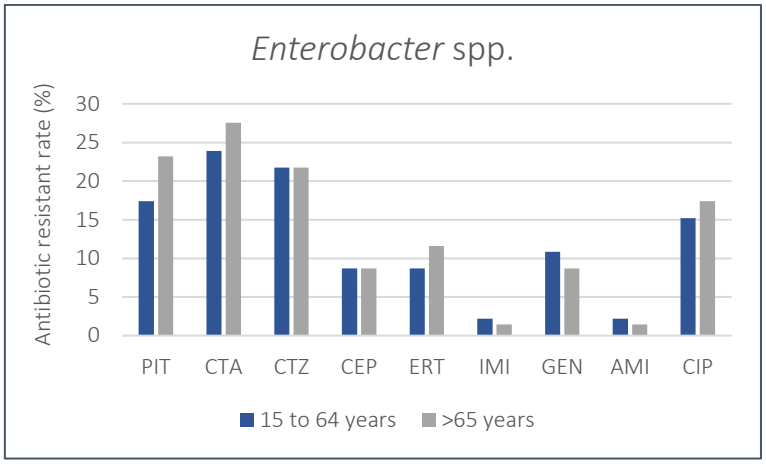


Figure 7. Antimicrobial resistance profiles of *Enterobacter* spp. by age groups. *No strain resistant to the antibiotics presented belonging to paediatric BSI was recovered. PIT: piperacillin-tazobactam, CTA: cefotaxime, CTZ: ceftazidime, CEP: cefepime, ERT: ertapenem, IMI: imipenem, GEN: gentamicin, AMI: amikacin, CIP: ciprofloxacin.*

Tables 19 and 20 show the susceptibility profiles of *K. oxytoca* and *P. mirabilis*, respectively. In a similar way to the rest of the *Enterobacterales*, the ESBL-producing isolates (four in the case of *K. oxytoca* and two in the case of *P. mirabilis*) occurred in adults, with mean ages of 75 and 88 years, respectively. Figures 8 and 9 depict the antimicrobial resistance profiles by age range.

Table 19. Antimicrobial non-resistance rates of *K. oxytoca*.

	PERCENT OF ANTIMICROBIAL NON-RESISTANCE										
	YEAR	TOTAL	AMC ≤ 8	PIT ≤ 8	CTA ≤ 2	CTZ ≤ 4	CEP ≤ 4	ERT ≤ 0.5	CIP ≤ 0.5	GEN ≤ 2	AMI ≤ 8
K. oxytoca	2015	16	93.8	93.8	87.5	100	100	100	93.8	93.8	100
	2016	12	75.0	83.3	100	100	100	100	100	100	100
	2017	17	94.1	94.1	100	100	100	100	94.1	100	100
	2018	19	94.7	94.7	94.7	100	100	100	89.5	100	100
	2019	13	69.2	76.9	92.3	100	100	100	92.3	92.3	100
	2020	16	100	100	100	100	100	100	100	100	100
	Total	93	89.2	91.4	95.7	100	100	100	94.6	97.8	100

AMC: amoxicillin-clavulanate, PIT: piperacillin-tazobactam, CTA: cefotaxime, CTZ: ceftazidime, CEP: cefepime, ERT: ertapenem, CIP: ciprofloxacin, GEN: gentamicin, AMI: amikacin.

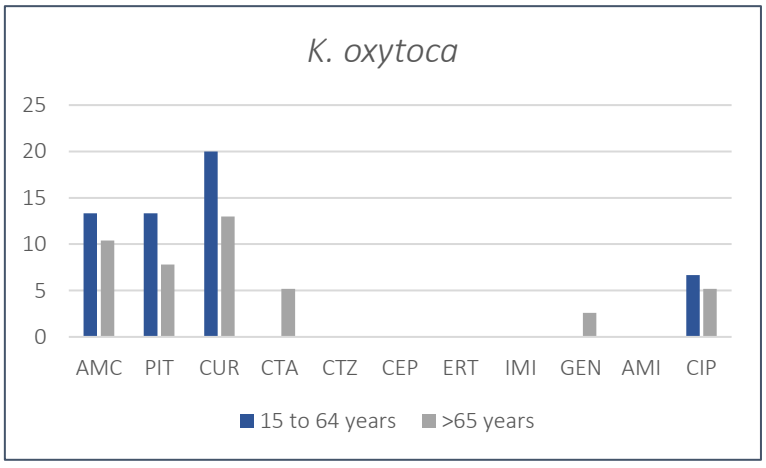


Figure 8. Antimicrobial resistance profiles of *K. oxytoca* by age groups. *No strain resistant to the antibiotics presented belonging to paediatric BSI was recovered. AMC: amoxicillin-clavulanate, PIT: piperacillin-tazobactam, CUR: cefuroxime, CTA: cefotaxime, CTZ: ceftazidime, CEP: cefepime, ERT: ertapenem, GEN: gentamicin, AMI: amikacin, CIP: ciprofloxacin.*

Table 20. Antimicrobial non-resistance rates of *P. mirabilis.*

	PERCENT OF ANTIMICROBIAL NON-RESISTANCE										
	YEAR	TOTAL	AMC ≤ 8	PIT ≤ 8	CTA ≤ 2	CTZ ≤ 4	CEP ≤ 4	ERT ≤ 0.5	CIP ≤ 0.5	GEN ≤ 2	AMI ≤ 8
P. mirabilis	2015	11	100	100	100	100	100	100	45.5	54.5	81.8
	2016	12	100	100	100	100	100	100	50.0	66.7	66.7
	2017	11	90.9	100	100	100	100	100	63.6	81.8	90.9
	2018	18	83.3	94.4	100	100	100	100	50.0	72.2	77.8
	2019	14	100	100	100	100	100	100	57.1	78.6	92.9
	2020	19	89.5	100	94.7	100	94.7	100	36.8	63.2	84.2
	Total	85	92.9	98.8	98.8	100	98.8	100	49.4	69.4	82.4

AMC: amoxicillin-clavulanate, PIT: piperacillin-tazobactam, CTA: cefotaxime, CTZ: ceftazidime, CEP: cefepime, ERT: ertapenem, CIP: ciprofloxacin, GEN: gentamicin, AMI: amikacin.

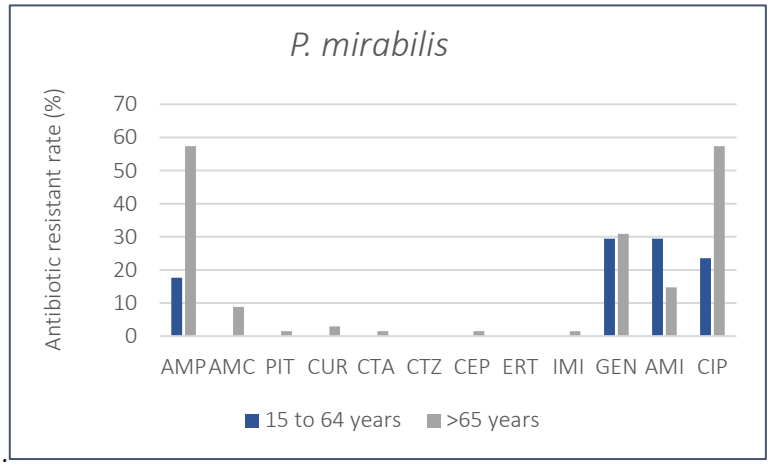


Figure 9. Antimicrobial resistance profiles of *P. mirabilis* by age groups. *No BSI episode produced by P. mirabilis was recovered in children. AMP: ampicillin, AMC: amoxicillin-clavulanate, PIT: piperacillin-tazobactam, CUR: cefuroxime, CTA: cefotaxime, CTZ: ceftazidime, CEP: cefepime, ERT: ertapenem, IMI: imipenem, GEN: gentamicin, AMI: amikacin, CIP: ciprofloxacin*.

For *P. aeruginosa*, 87.0% and 87.6% displayed MICs of ≤ 16 mg/L of piperacillin-tazobactam and ≤ 8 mg/L of ceftazidime, respectively. 72.0% yielded meropenem MICs ≤ 2 mg/L and 94.7% MICs ≤ 8 mg/L; 72.0% yielded imipenem MICs of ≤ 4 mg/L (Table 21).

The MDR phenotype was present in 8.8% of the *P. aeruginosa* isolates, with incidences/1000 admissions ranging from 0.02 to 0.2; all isolates belonged to the HUA. No significant increase in the annual incidence of MDR was observed.

No BSI episode produced by MDR *P. aeruginosa* occurred in children. The mean age was 65 years, and practically half of the episodes occurred in men (52.9%).

The antimicrobial resistance profiles of *P. aeruginosa* by age range is shown in Figure 10.

Concerning *A. baumannii*, all three isolates displayed amikacin, imipenem, and ciprofloxacin MICs of ≤ 8 mg/L, ≤ 2mg/L and ≤ 1 mg/L, respectively.

Table 21. Antimicrobial non-resistance rates of *P. aeruginosa*.

			PERCENT OF ANTIMICROBIAL NON-RESISTANCE							
	YEAR	TOTAL	PIT ≤ 16	CTZ ≤ 8	CEP ≤ 8	MER ≤ 8	IMI ≤ 4	CIP ≤ 0.5	TOB ≤ 2	AMI ≤ 16
P. aeruginosa	2015	18	94.4	88.9	88.9	100	72.2	72.2	72.2	94.4
	2016	36	75.0	72.2	75.0	94.4	55.6	52.8	58.3	94.4
	2017	37	86.5	89.2	81.1	89.2	64.9	54.1	59.5	97.3
	2018	30	76.7	90.0	90.0	100	56.7	60.0	63.3	96.7
	2019	34	94.1	97.1	91.2	100	94.1	73.5	79.4	94.1
	2020	38	97.4	89.5	94.7	94.7	86.8	71.1	84.2	92.1
	Total	193	87.0	87.6	86.5	95.9	72.0	63.2	69.4	94.8

PIT: piperacillin-tazobactam, CTZ: ceftazidime, CEP: cefepime, MER: meropenem, IMI: imipenem, CIP: ciprofloxacin, TOB: tobramycin, AMI: amikacin.

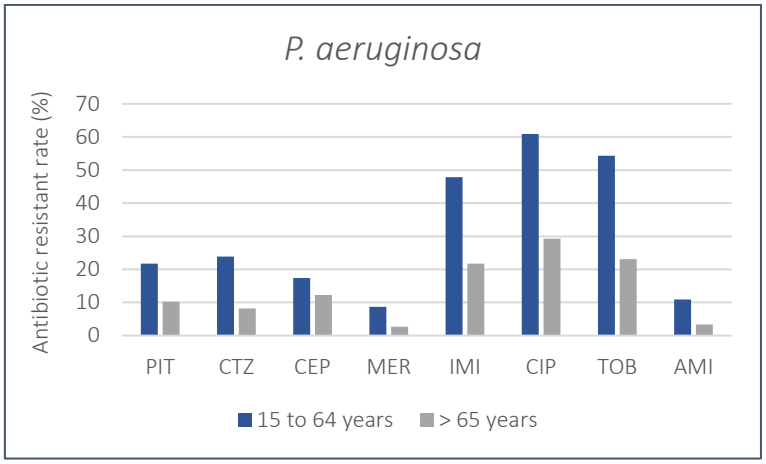


Figure 10. Antimicrobial resistance profiles of *P. aeruginosa* by age groups. *No BSI episode produced by P. aeruginosa was recovered in children. PIT: piperacillin-tazobactam, CTZ: ceftazidime, CEP: cefepime, MER: meropenem, IMI: imipenem, TOB: tobramycin, CIP: ciprofloxacin, AMI: amikacin.*

Gram-positive microorganisms

The overall MRSA percentage was 19.4%. The incidence of MRSA was 0.4 and 0.2 episodes/1000 admissions in HUA and in HAD, no significant trends were observed. Figure 11 depicts the proportion of MRSA and methicillin-susceptible *S. aureus* (MSSA) isolates.

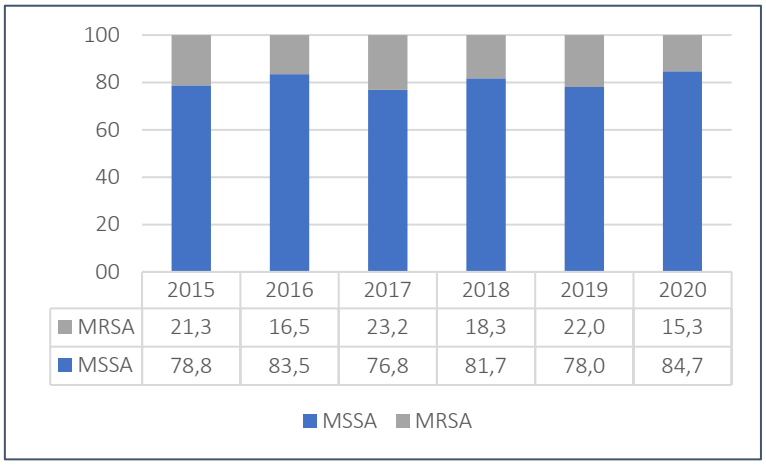


Figure 11. Proportion of MSSA and MRSA BSI from 2015 to 2020. *MSSA: methicillin-susceptible Staphylococcus aureus, MRSA: methicillin-resistant Staphylococcus aureus.*

None of the MRSA isolates was recovered from children. The mean age of the patients with BSI caused by MRSA (74 years) was higher than that of the episodes produced by MSSA (65 years) and most of the episodes (71.2%) occurred in men.

MRSA strains were associated with greater resistance rates to other antimicrobials than MSSA strains. Only one MRSA isolate displayed vancomycin and teicoplanin MICs of above 2 mg/L (4 mg/L). All *S. aureus* isolates yielded linezolid and daptomycin MICs of

below 4 mg/L and 1 mg/L, respectively. Trimethoprim-sulfamethoxazole MICs were ≤ 2 mg/L in 99.6% (98.9% in MRSA strains) (Table 22).

The antimicrobial resistance profiles of *S. aureus* in adult and paediatric BSIs are depicted in Figure 12.

Table 22. Antimicrobial non-resistance rates of *S. aureus*.

				PERCENT OF ANTIMICROBIAL NON-RESISTANCE								
		YEAR	TOTAL	VAN ≤ 2	TEI ≤ 2	LIN ≤ 4	DAP ≤ 1	TRS ≤ 4	CIP ≤ 1	GEN ≤ 1	TOB ≤ 1	TET ≤ 2
S. aureus	Total (485)	2015	80	100	100	100	100	98.8	73.8	85.0	83.8	96.3
		2016	85	100	100	100	100	100	75.3	81.2	78.8	98.8
		2017	82	98.8	98.8	100	100	100	73.2	80.5	75.6	92.7
		2018	71	100	100	100	100	98.6	69.0	88.7	78.9	94.4
		2019	82	100	100	100	100	100	70.7	81.7	76.8	95.1
		2020	85	100	100	100	100	100	80.0	81.2	80.0	96.5
		Total	485	99.8	99.8	100	100	99.6	73.8	82.9	79.0	95.7
	MRSA (94)	2015	17	100	100	100	100	100	5.9	82.4	41.2	100
		2016	14	100	100	100	100	100	0	85.7	50.0	100
		2017	19	94.7	94.7	100	100	100	10.5	57.9	52.6	94.7
		2018	13	100	100	100	100	92.3	7.7	76.9	53.8	100
		2019	18	100	100	100	100	100	11.1	72.2	44.4	94.4
		2020	13	100	100	100	100	100	38.5	84.6	61.5	84.6
		Total	94	98.9	98.9	100	100	98.9	11.7	75.5	50.0	95.7

MRSA: methicillin-resistant Staphylococcus aureus, VAN: vancomycin, TEI: teicoplanin, LIN: linezolid, DAP: daptomycin, TRS: trimethoprim-sulfamethoxazole, CIP: ciprofloxacin, GEN: gentamicin, TOB: tobramycin, TET: tetracycline.

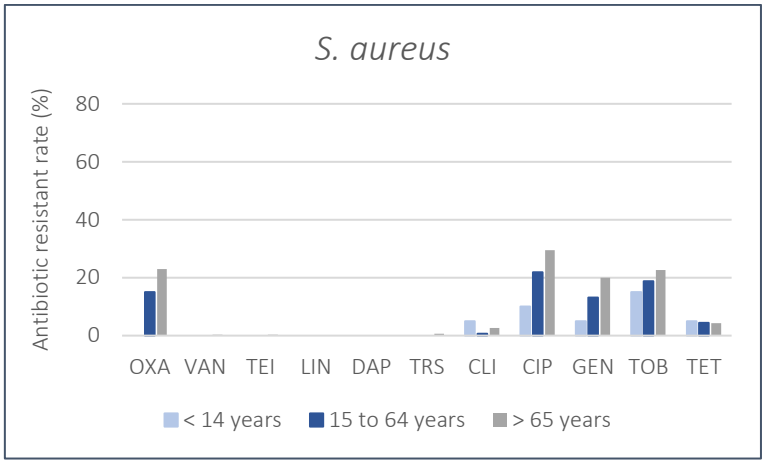


Figure 12. Antimicrobial resistance profiles of *S. aureus* by age groups. *OXA: oxacillin, VAN: vancomycin, TEI: teicoplanin, LIN: linezolid, DAP: daptomycin, TRS: trimethoprim-sulfamethoxazole, CLI: clindamycin, CIP: ciprofloxacin, GEN: gentamicin, TOB: tobramycin, TET: tetracycline.*

For *S. epidermidis*, all strains yielded vancomycin MICs $\leq$ 4 mg/L. Linezolid susceptibility rates evolved from 95.8% in 2015 to 75.0% in 2020, but the decrease in linezolid susceptibility rates throughout the study period was not statistically significant (Table 23).

Table 23. Antimicrobial non-resistance rates of *S. epidermidis.*

	PERCENT OF ANTIMICROBIAL NON-RESISTANCE										
	YEAR	TOTAL	VAN ≤ 4	TEI ≤ 4	LIN ≤ 4	DAP ≤ 1	TRS ≤ 4	CIP ≤ 1	GEN ≤ 1	TOB ≤ 1	TET ≤ 2
S. epidermidis	2015	71	100	100	95.8	100	81.7	45.1	64.8	50.7	80.3
	2016	64	100	98.4	95.3	100	67.2	35.9	40.6	32.8	87.5
	2017	70	100	97.1	95.7	100	71.4	41.4	47.1	35.7	88.6
	2018	77	100	100	93.5	100	88.3	42.9	59.7	48.1	87.0
	2019	101	100	100	91.1	100	89.1	41.6	49.5	37.6	87.1
	2020	84	100	98.8	75.0	100	75.0	28.6	35.7	28.6	86.9
	Total	467	100	99.1	90.6	100	79.7	39.2	49.5	38.8	86.3

VAN: vancomycin, TEI: teicoplanin, LIN: linezolid, DAP: daptomycin, TRS: trimethoprim-sulfamethoxazole, CIP: ciprofloxacin, GEN: gentamicin, TOB: tobramycin, TET: tetracycline.

The antimicrobial resistance profiles of *S. epidermidis* in adult and paediatric BSIs are depicted in Figure 13.

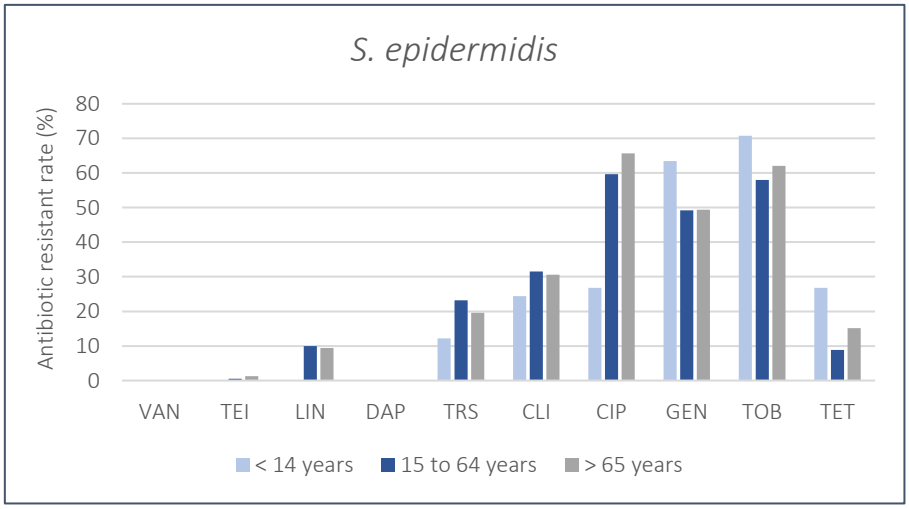


Figure 13. Antimicrobial resistance profiles of *S. epidermidis* by age groups. *VAN: vancomycin, TEI: teicoplanin, LIN: linezolid, DAP: daptomycin, TRS: trimethoprim-sulfamethoxazole, CLI: clindamycin, CIP: ciprofloxacin, GEN: gentamicin, TOB: tobramycin, TET: tetracycline.*

Regarding *E. faecalis*, all strains were susceptible to teicoplanin (≤ 2 mg/L) and all except one isolated in 2016, and another isolated in 2017 were susceptible to vancomycin (≤ 4 mg/L) and to linezolid (≤ 4 mg/L), respectively (Table 24). The vancomycin-resistant *E. faecalis* exhibited the VanB phenotype.

Table 24. Antimicrobial susceptibility rates of *E. faecalis*.

			PERCENT OF ANTIMICROBIAL SUSCEPTIBILITY				
	YEAR	TOTAL	VAN ≤ 4	TEI ≤ 2	LIN ≤ 4	LEV ≤ 4	DAP ≤ 4
E. faecalis	2015	30	100	100	100	53.3	100
	2016	45	97.8	100	100	68.9	100
	2017	37	100	100	97.3	67.6	100
	2018	36	100	100	100	72.2	100
	2019	29	100	100	100	86.2	100
	2020	39	100	100	100	64.1	100
	Total	216	99.5	100	99.5	68.5	100

VAN: vancomycin, TEI: teicoplanin, LIN: linezolid, LEV: levofloxacin, DAP: daptomycin.

All *E. faecium* isolates displayed MICs below 4 mg/L for both vancomycin and linezolid and MICs lower than 2 mg/L for teicoplanin (Table 25).

Table 25. Antimicrobial susceptibility rates of *E. faecium*.

			PERCENT OF ANTIMICROBIAL SUSCEPTIBILITY				
	YEAR	TOTAL	VAN ≤ 4	TEI ≤ 2	LIN ≤ 4	LEV ≤ 4	DAP ≤ 8
E. faecium	2015	29	100	100	100	17.2	100
	2016	24	100	100	100	12.5	100
	2017	26	100	100	100	19.2	100
	2018	29	100	100	100	10.3	100
	2019	25	100	100	100	16.0	100
	2020	34	100	100	100	17.6	100
	Total	167	100	100	100	15.6	100

VAN: vancomycin, TEI: teicoplanin, LIN: linezolid, LEV: levofloxacin, DAP: daptomycin.

For the *S. pneumoniae* isolates, penicillin (≤ 0.06 mg/L) and cefotaxime (≤ 0.5 mg/L) susceptibility rates were 80.9% and 96.7%, respectively.

All *S. pyogenes* isolates were penicillin susceptible (≤ 0.25 mg/L).

Anaerobic bacteria

For BFG, amoxicillin-clavulanate, piperacillin-tazobactam, imipenem, and metronidazole showed non-resistance rates of 92.1%, 96.5%, 98.2 and 97.4%, respectively. Amoxicillin-clavulanate resistance rates significantly increased over the study period (*P*=0.043) (Table 26).

Table 26. Antimicrobial non-resistance rates of BFG.

			PERCENT OF ANTIMICROBIAL NON -RESISTANCE			
	YEAR	TOTAL	AMC ≤ 8	PIT ≤ 16	IMI ≤ 4	MET ≤ 4
BFG	2015	24	95.8	100	100	95.8
	2016	19	94.7	100	100	100
	2017	25	100	100	100	100
	2018	15	93.3	93.3	100	100
	2019	13	84.6	92.3	100	84.6
	2020	18	77.8	88.9	88.9	100
	Total	114	**92.1**	96.5	98.2	97.4

In bold, significant trend (P<0.05). *BFG: Bacteroides fragilis Group, AMC: amoxicillin-clavulanate, PIT: piperacillin-tazobactam, IMI: imipenem, MET: metronidazole.*

Yeasts

C. albicans and *C. parapsilosis* remained highly susceptible to the main antifungals used in clinical practice (Table 27). *C. glabrata* has intrinsically low susceptibility to azoles and 10.3% of the strains were resistant to amphotericin B.

Table 27. Antifungal non-resistance rates of *Candida albicans*, *C. glabrata* and *C. parapsilosis*.

Organism	YEAR	TOTAL	PERCENT OF ANTIFUNGAL NON-RESISTANCE						
			ITC ≤ 0.06	VRC ≤ 0.25	POS ≤ 0.06	AMB ≤ 1	FLC ≤ 4	MFG ≤ 0.016	AFG ≤ 0.03
C. albicans	2015	13	100	100	100	100	100	100	100
	2016	8	100	100	100	100	100	100	100
	2017	7	100	100	100	100	100	100	71.4
	2018	6	100	100	100	100	100	66.7	66.7
	2019	5	80.0	100	100	100	100	100	60.0
	2020	10	100	100	100	100	100	100	80.0
	Total	49	98.0	100	100	100	100	100	83.7
C. glabrata	YEAR	TOTAL	AMB ≤ 1	FLC ≤ 16	MFG ≤ 0.03	AFG ≤ 0.06			
	2015	7	85.7	57.1	100	100			
	2016	7	85.7	71.4	100	85.7			
	2017	8	87.5	75.0	87.5	87.5			
	2018	0	0	0	0	0			
	2019	2	100	100	100	100			
	2020	5	100	100	100	100			
	Total	29	89.7	57.1	96.6	93.1			
C. parapsilosis	YEAR	TOTAL	ITC ≤ 0.125	VRC ≤ 0.25	POS ≤ 0.06	AMB ≤ 1	FLC ≤ 4	MFG ≤ 2	AFG ≤ 4
	2015	2	100	100	100	100	100	100	100
	2016	8	100	100	100	100	100	100	100
	2017	3	100	100	100	100	100	100	100
	2018	2	100	100	100	100	100	100	100
	2019	3	100	100	100	100	100	100	100
	2020	3	100	100	100	100	100	100	100
	Total	21	100	100	100	100	100	100	100

ITC: itraconazole, VRC: voriconazole, POS: posaconazole, AMB: amphotericin B, FLC: fluconazole, MFG: micafungin, AFG: anidulafungin.

OBJECTIVE 2

Evaluation of two assays using E-tests and VITEK®-2 for rapid detection and accurate differentiation of bacterial antibiotic susceptibility in *Enterobacterales*

Summary

The objective of this study was to evaluate two assays using E-tests and VITEK®-2 for rapid detection and accurate differentiation of bacterial antibiotic susceptibility in *Enterobacterales* in order to design a strategy to be able to report reliable preliminary antibiograms in the shortest possible time. One of these strategies could be implemented if the Department of Microbiology is granted extended hours of operation.

A total of 121 prospective BC positive samples isolated from March to December 2019 showing Gram-negative enteric bacilli were evaluated.

After 5h, 95.0% had readable E-test MICs, and after 7 h 99.2%. For *E. coli* the MIC could be read after 5h in all cases except for one. For the VITEK®-2 assay, most MIC results were available after 7 hours.

For both methods under study, the lowest CA was obtained for amoxicillin-clavulanate. Achieved data showed 0.8% VME for the E-test-based method. In the VITEK®-2 assay the highest VME rate was 3.3% for gentamicin.

The following species were included in this study: *E. coli* (96), *K. pneumoniae* (7), *P. mirabilis* (6), *Raoultella ornithinolytica* (4), *K. aerogenes* (3), *K. variicola* (2), *Enterobacter* spp. (2), *K. oxytoca* (1).

Concerning the E-test based method, after 5 h, 95.0% had readable MICs, and after 7 h 99.2%. Species for which the MIC could not be read after 5 hours included *P. mirabilis* (83.3%) and an ESBL-harbouring *E. coli* isolate. For *E. coli* the MIC could be read at 5 hours in all cases except for one.

For the VITEK®-2 assay, most MIC results were available after 7h of incubation with the exception of imipenem and piperacillin-tazobactam. For these antibiotics only less than half and less than 10% of the strains analysed had MICs available after 7 hours, respectively. MICs available at each time and for each antimicrobial agent are shown in Table 28. The mean final time was 9.2h.

Table 28. Percentage of isolates with available MICs at different times of incubation.

	MIC AVAILABLE AT (%)	
	5 h	7 h
AMC	97.5	100
PIT	0	7.4
CTA	53.7	99.2
CTZ	31.4	93.3
CEP	79.3	99.2
IMI	1.7	44.6
ERT	43.8	99.2
CIP	95.9	100
GEN	94.2	100
AMI	95.0	100

MIC: minimum inhibitory concentration, AMC: amoxicillin-clavulanate, PIT: piperacillin-tazobactam, CTA: cefotaxime, CTZ, ceftazidime, CEP, cefepime, IMI: imipenem, ERT: ertapenem, CIP: ciprofloxacin, GEN: gentamicin, AMI: amikacin.

Comparison between MIC interpretations obtained with rapid direct E-test after 5 and 7h of incubation, the VITEK®-2 assay, and the reference method is reported in Table 29.

CA were higher than 87.6% for the E-test method and higher than 88.4% for the VITEK®-2 assay. For both methods under study, the lowest CA was obtained for amoxicillin-clavulanate. In contrast, CA for cephalosporins and carbapenems was high for both assays, showing ≥ 96.7% agreement.

For the E-test method, eight, three and six ME were detected for amoxicillin-clavulanate, piperacillin-tazobactam and amikacin, respectively. In the case of the Vitek®-2 method,

the highest ME rate was also obtained for amoxicillin clavulanate (11.6%) and piperacillin-tazobactam (6%).

Overall, achieved data showed 1/121 (0.8 %) VME for the E-test-based method. In the VITEK®-2 assay the highest VME rate was 3.3% for the antibiotic gentamicin.

Table 29. Agreement of direct rapid E-test (5–7 h) and the VITEK®-2 assay with the reference method.

	E-TEST 5 HOURS					E-TEST 7 HOURS					VITEK®-2				
	CA	mE	ME	VMEs	NR	CA	mE	ME	VMEs	NR	CA	mE	ME	VMEs	NR
AMC	87.6 (106)	0	6.6 (8)	0.8 (1)	5.0 (6)	87.6 (106)	0	10.7 (13)	0.8 (1)	0.8 (1)	88.4 (107)	0	11.6 (14)	0	0
PIT	94.2 (114)	0	2.5 (3)	0.8 (1)	2.5 (3)	95.0 (115)	0	3.3 (4)	0.8 (1)	0.8 (1)	93.4 (113)	0	5.0 (6)	0	1.7 (2)
CTA	96.7 (117)	0	0	0.8 (1)	2.5 (3)	99.2 (120)	0	0	0	0.8 (1)	100 (121)	0	0	0	0
CTZ	-	-	-	-	-	-	-	-	-	-	100 (121)	0	0	0	0
CEP	-	-	-	-	-	-	-	-	-	-	99.2 (120)	0.8 (1)	0	0	0
MER	97.5 (118)				2.5 (3)	99.2 (120)				0.8 (1)	100 (121)	0	0	0	0
IMI	-	-	-	-	-	-	-	-	-	-	98.3 (118)	0	0	0	1.7 (2)
ERT	-	-	-	-	-	-	-	-	-	-	100 (121)	0	0	0	0
CIP	94.2 (114)	3.3 (4)	0	0	2.5 (3)	96.7 (117)	2.5 (3)	0	0.8 (1)	0	94.2 (114)	5.0 (6)	0	0.8 (1)	0
GEN	-	-	-	-	-	-	-	-	-	-	95.0 (115)	0	1.7 (2)	3.3 (4)	0
AMI	92.6 (112)	0	5.0 (6)	0.8 (1)	1.7 (2)	93.4 (113)	0	5.0 (6)	0.8 (1)	0.8 (1)	97.5 (118)	0	0.8 (1)	1.7 (2)	0

MIC: minimum inhibitory concentration, CA: categorical agreement, mE: minor error, ME: major error, VME: very major error, NR: not readable, AMC: amoxicillin-clavulanate, PIT: piperacillin-tazobactam, CTA: cefotaxime, CTZ, ceftazidime, CEP, cefepime, MER: meropenem, IMI: imipenem, ERT: ertapenem, CIP: ciprofloxacin, GEN: gentamicin, AMI: amikacin.

OBJECTIVE 3

Analysis of the time-to-positivity of blood cultures during a two-year period: evaluation of real and contaminated episodes

Summary

This study aimed to: i) evaluate the TTP of all bacteraemia episodes (true and contaminations) isolated during a two-year period (2019-2020) to study to what extent the TTP can provide information on the type of microorganism isolated and its involvement in infection, ii) analyse those episodes in which the TTP was greater than 24 h, iii) analyse separately all candidaemia episodes and determine the relationship between the TTP and 28-day mortality, and iv) explore the influence of the TTP and the PBDP in BCC by CoNS using BLR analysis and achieve a predicting formula.

The median TTP for all true BSI episodes was 12.1 (IQR, 10.1-16.8) h; 49.4% grew in $\leq$ 12 h, and 87.3% grew in $\leq$ 24 h. Bottles of true episodes flagged positive considerably faster (P<0.0001) than the ones considered contaminations. The probability of true BSI was 98.1% (95% CI, 96.9-98.9%) when the TTP was $\leq$ 12 h, and 71.3% (95% CI, 69.4-73.3%) when the TTP was $\leq$ 24 h. When a flask signalled positive in the 24-48 h TTP range the probability of contamination was of 69.7% (95% CI, 65.8-73.8%). A BLR formula was proposed to predict the probability of contamination from TTP and PBDP and was fitted in Excel to allow the user play with the variables.

Study population characteristics

A total of 1784 true episodes (1669 monomicrobial and 115 polymicrobial), and of 1106 contaminations was evaluated.

Figure 14 shows the total distribution of the TTP within the whole cohort.

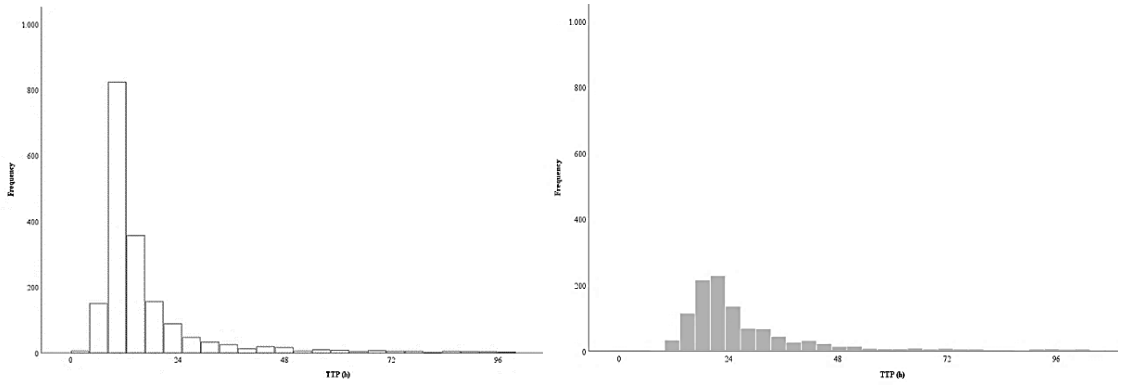


Fig.14 A. Real BSI episodes. Fig. 14 B. Contaminated episodes.

Figure 14. Distribution of the TTP of real BSI episodes (14 A) and contaminated episodes (14 B). *BSI: bloodstream infection, TTP: Time-to-positivity.*

For the true episodes, mean age was 70 years and 1125 were male (63.1%), and in the contamination group mean age was 65 years and 674 (60.9%) were male.

In children (≤ 14 years), 28 episodes were classified as real BSI (64.3% were ≤ 1 years old), and 40 were considered contaminations.

Figure 15 depicts the distribution of BC requesting departments and the ED discharge destinations in 2019 and 2020.

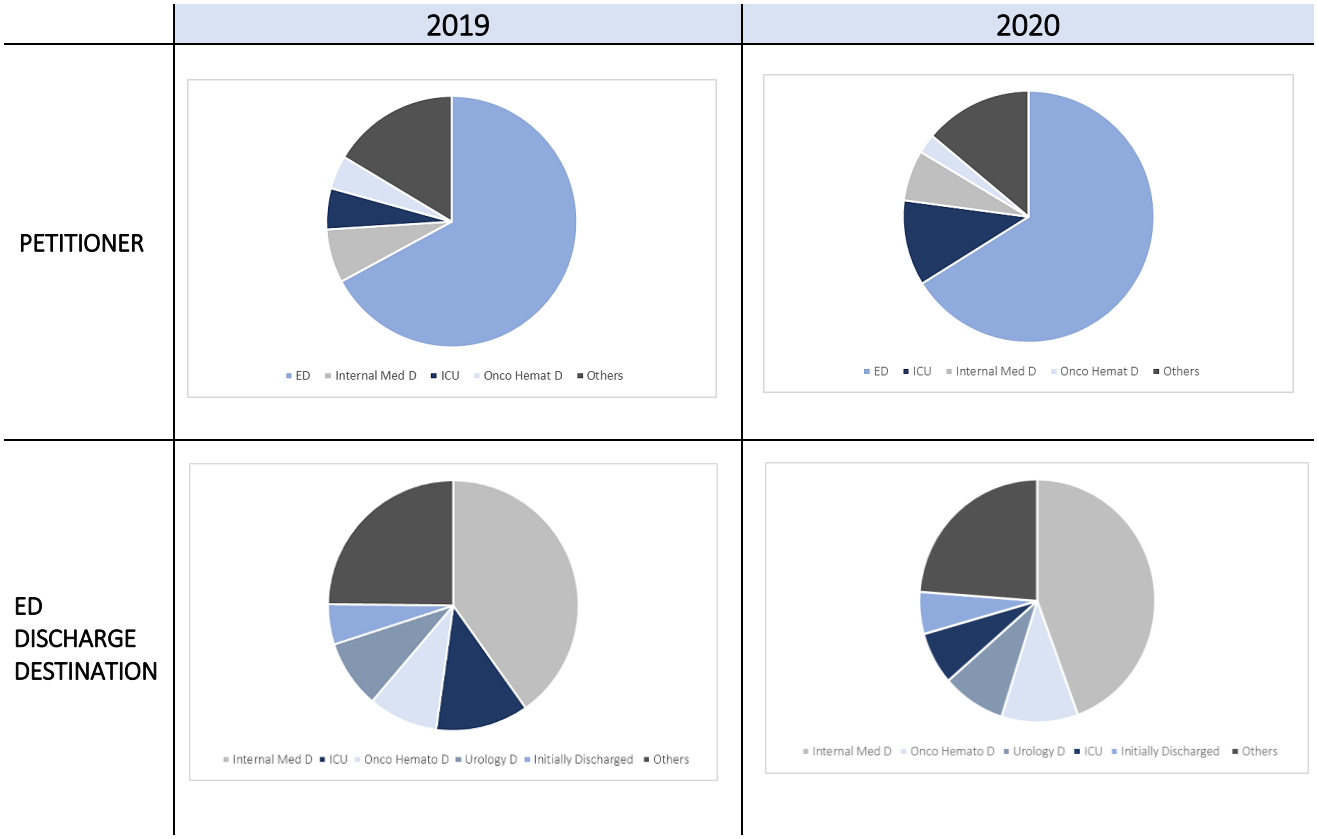


Figure 15. Distribution of BC requesting departments and the ED discharge destinations in 2019 and 2020. *ED: Emergency Department, ICU: Intensive care unit.*

In 2019, 893 episodes were considered; 67.1% of the episodes belonged to BCs ordered in the ED, 6.9% to BCs ordered in the Internal Medicine Department, 5.3% belonged to the ICU, and 4.3% to Oncohaematological Departments. Most of the patients whose BCs had been requested in the ED were admitted to the Internal Medicine Department (40.2%), the ICU (12.0%), Oncohaematological Departments (9.0%) and the Urology Department (8.8 %); 5.2% of the patients whose BCs had been ordered in the ED were initially discharged.

In 2020, 891 episodes were considered; 66.1% of the episodes belonged to BCs ordered in the ED, 11.0% belonged to BCs requested in the ICU, 6.5% to BCs ordered in the Internal Medicine Department, and 2.8% to Oncohaematological Departments. Most of the patients whose BCs had been requested in the ED were admitted to the Internal Medicine Department (44.8%), Oncohaematological Departments (10.5%), the Urology Department (8.7 %), and the ICU (7.0%); 5.6% of the patients whose BCs had been ordered in the ED were initially discharged.

Regarding the episodes considered contaminations, 518 of these episodes were obtained in 2019 and 588 in 2020. In both years, most episodes belonged to BCs drawn in the ED (71.0% in 2019 and 62.2% in 2020), followed by the ICU (10.4% in 2019 and 19.0% in 2020).

The ratio real BSI episode versus contamination is depicted in Figure 16.

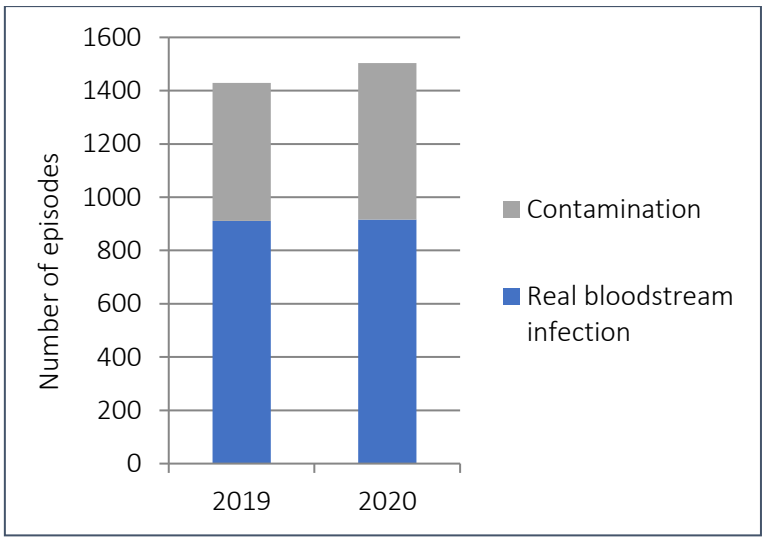


Figure 16. Ratio real BSI episodes versus contamination.

In most of the real episodes, four or more BC bottles flagged positive (40.6%); only one vial was positive in 26.8% of the episodes, two vials in 20.9% of the episodes, and three in 11.7%. In contrast, in most of the contaminated episodes, only one BC bottle became positive (77.6%); two vials yielded positive in 20.3% of the episodes, three in 1.4%, and four in 0.7%.

A. True BSI episodes

Table 30 summarizes the demographic characteristics of the 1784 true BSI episodes.

Table 30. Demographic characteristics of the 1784 BSI episodes.

	n (%)	MEAN TTP (h)	MEDIAN TTP (IQR) (h)	MIN TTP (h)	MAX TTP (h)
AGE GROUP					
≤ 1	18 (1.0)	13.8	10.5 (9.3-17.7)	6.1	37.4
2-14	10 (0.6)	29.6	18.7 (15.0-38.5)	13.1	72.2
15-64	541 (30.3)	17.0	12.4 (10.4-17.7)	3.0	117.6
≥ 65	1215 (68.1)	16.0	11.8 (10.0-16.1)	2.9	159.0
SEX					
Male	1125 (63.1)	17.0	12.2 (10.1-17.5)	2.9	159.0
Female	659 (36.9)	15.3	11.7 (10.1-15.6)	2.9	98.0
NUMBER OF MICROORGANISMS INVOLVED					
Monomicrobial	1669 (93.6)	16.6	12.1 (10.1-17.1)	2.9	159.0
Polymicrobial	115 (6.4)	13.6	11.3 (9.1-14.5)	4.9	62.3
TOTAL	1784 (100)	16.4	12.1 (10.1-16.7)	2.9	159.0

n: number, BSI: bloodstream infection, TTP: time-to-positivity, IQR: interquartile range, MIN: minimum, MAX: maximum.

No significant differences in the TTP were found between children and adults (*P*=0.375). Nevertheless, increasing age was associated with decreasing TTP (Spearman's correlation = -0.076; *P*=0.001).

Median TTP for all true BSI episodes was 12.1 (IQR, 10.1-16.8) h; median TTP for the monomicrobial episodes was 12.1 (IQR, 10.1-17.1) h, and for the polymicrobial episodes was 11.3 (IQR, 9.1-14.5) h. TTP for polymicrobial episodes was significantly shorter than for monomicrobial BSI (*P*=0.005).

Nearly half of the episodes (49.4%, 882/1784) grew in $\leq$ 12 h, 87.3% (1558/1784) in $\leq$ 24 h and 96.2% (1726/1784) in $\leq$ 48 h.

The probability of true BSI was 98.1% (95% CI, 96.9-98.9%) when the TTP was $\leq$ 12 h, and 71.3% (95% CI, 69.4-73.3%) when the TTP was $\leq$ 24 h. However, when the TTP > 48 h, the probability of true bacteraemia decreased to 37.6% (95% CI, 30.6-45.1%).

A.1. TTP analysis by type of microorganism

Figure 17 depicts the distribution of TTP by type of microorganism of the main groups of microorganisms isolated, and Figure 18 depicts the percentage of growth by TTP ranges and by type of microorganism.

Overall, the longest TTP was 159 h in a case of spondylodiscitis caused by *C. acnes*, and the shortest TTP was 2.9 h in a case of severe pneumonia caused by *S. pneumoniae* and in one of cholelithiasis caused by *E. coli*.

For BSI caused by bacteria median TTP was 12.0 (IQR, 10.1-16.2) h, while median TTP of fungaemia was 40.3 (IQR, 32.5-61.0) h (P<0.0001).

By groups, *Candida* spp. (median TTP of 37.9 h) and strict anaerobes (median time of 25.3 h) yielded the longest TTP and *Enterobacterales* (median TTP of 11.1 h), and *Streptococcus* spp. (median TTP of 11.2 h) the shortest.

Strict anaerobic bacteria grew significantly slower than other bacteria (P<0.0001), and excluding strict anaerobes, Gram-negative bacteria grew significantly faster than Gram-positive ones (P<0.0001).

Table 31 outlines the TTP characteristics by type of microorganism.

The characteristics of the TTPs by groups of microorganisms will be analysed in detail below.

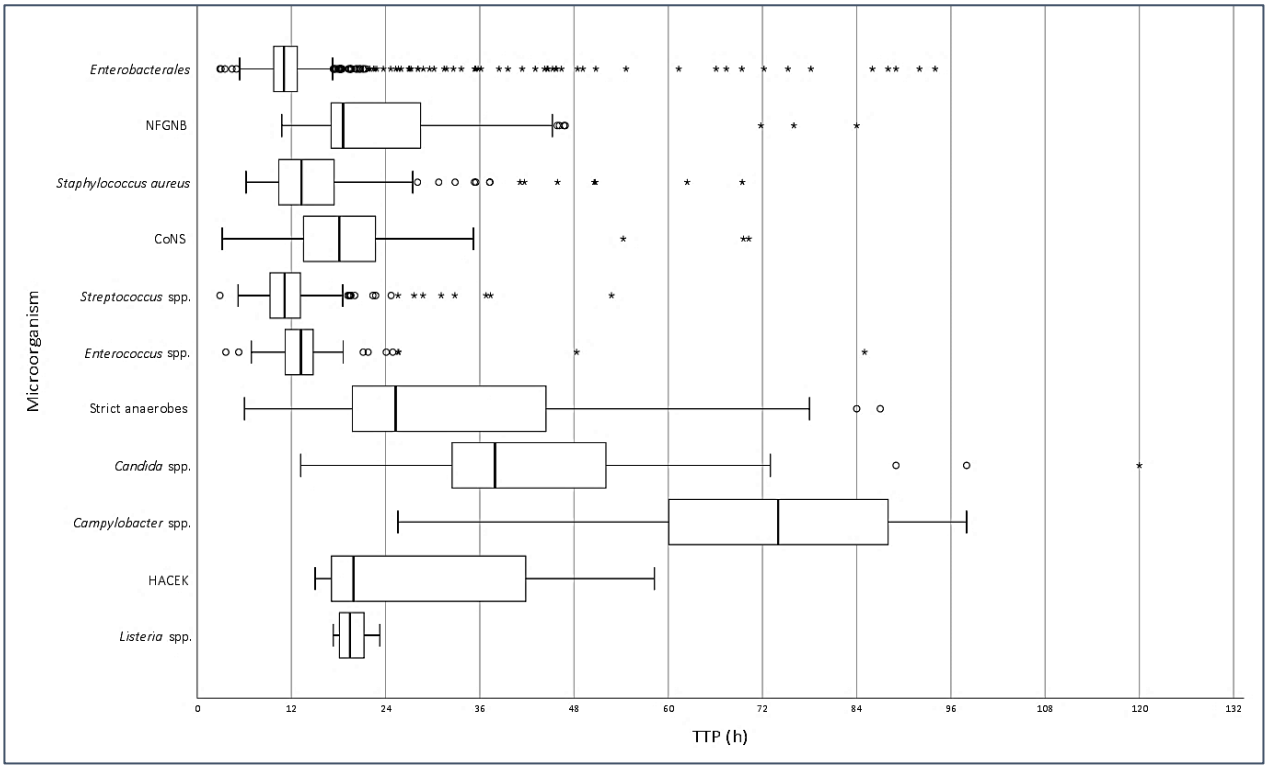


Figure 17. Box plot graph of the TTP by type of microorganism. Circles represent outliers; asterisks represent extreme values. *TTP: time-to-positivity. NFGNB: Non-fermenting Gram-negative bacilli, CoNS: coagulase-negative staphylococci, HACEK*: *Haemophilus, Aggregatibacter, Cardiobacterium, Eikenella and Kingella.*

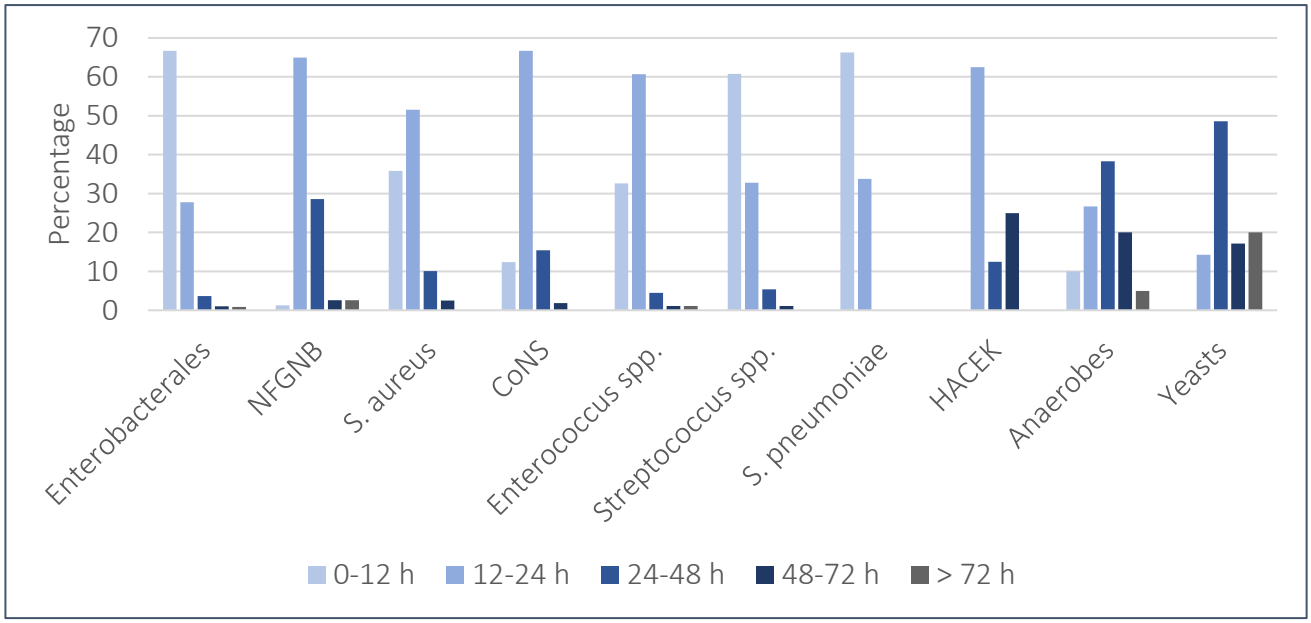


Figure 18. Percentage of growth by TTP ranges and by type of microorganism. *NFGNB: Non-fermenting Gram-negative bacilli, CoNS: coagulase-negative staphylococci, HACEK: Haemophilus, Aggregatibacter, Cardiobacterium, Eikenella and Kingella.*

Table 31. TTP by type of microorganism of true bacteraemia episodes.

	n	MEAN TTP (h)	MEDIAN TTP (IQR) (h)	MIN TTP (h)	MAX TTP (h)	≤ 12 h	12-24 h	24-48 h	48-72h	> 72h
Total	1784	16.4	12.1 (10.1-16.7)	2.9	159.0	49.4 (882)	37.9 (676)	8.9 (158)	2.3 (41)	1.5 (27)
Monomicrobial	1669	16.6	12.1 (10.1-17.1)	2.9	159.0	49.0 (817)	38.0 (635)	9.1 (152)	2.3 (38)	1.6 (27)
Polymicrobial	115	13.6	11.3 (9.1-14.5)	4.9	62.3	56.5 (65)	35.7 (41)	5.2 (6)	2.6 (3)	0
Enterobacterales	890	13.2	11.1 (9.8-12.8)	2.9	94.0	66.3 (590)	28.4 (253)	3.5 (31)	0.9 (8)	0.9 (8)
E. coli	634	12.4	10.7 (9.7-12.2)	2.9	94.0	70.8 (449)	25.6 (162)	2.2 (14)	0.6 (4)	0.8 (5)
Klebsiella spp.	133	14.3	11.3 (10.0-13.0)	5.6	92.0	64.7 (86)	25.6 (34)	8.3 (11)	0.8 (1)	0.8 (1)
Enterobacter spp.	40	15.2	11.6 (9.2-14.3)	5.9	86.0	57.5 (23)	35.0 (14)	2.5 (1)	2.5 (1)	2.5 (1)
NFGNB	72	25.0	18.6 (17.1-28.5)	10.8	84.0	1.4 (1)	66.7 (48)	27.8 (20)	1.4 (1)	2.8 (2)
P. aeruginosa	58	25.0	18.5 (16.9-27.0)	12.4	84.0	0	69.0 (40)	25.9 (15)	1.7 (1)	3.4 (2)
S. aureus	155	16.2	13.3 (10.3-17.7)	6.2	69.4	36.1 (56)	52.3 (81)	9.0 (14)	2.6 (4)	0
CoNS	142	19.4	18.1 (13.6-22.7)	3.2	70.3	14.1 (20)	67.6 (96)	16.2 (23)	2.1 (3)	0
S. epidermidis	122	19.9	18.3 (14.5-23.0)	3.2	70.3	12.3 (15)	68.0 (83)	17.2 (21)	2.5 (3)	0
***Streptococcus* spp.**	183	12.5	11.2 (9.3-13.2)	2.9	52.8	61.2 (112)	33.9 (62)	4.4 (8)	0.5 (1)	0
S. pneumoniae	77	11.0	11.2 (9.5-12.4)	2.9	17.6	64.9 (50)	35.1 (27)	0	0	0
***Enterococcus* spp.**	89	14.6	13.2 (11.2-14.8)	3.7	85.0	32.6 (29)	60.7 (54)	4.5 (4)	1.1 (1)	1.1 (1)
Strict anaerobes	57	33.2	253 (19.8-44.4)	6.0	87.0	10.5 (6)	26.3 (15)	40.4 (23)	17.5 (10)	5.3 (3)
***Candida* spp.**	33	44.9	37.9 (32.5-52.1)	13.2	120.0	0	15.2 (5)	51.5 (17)	18.2 (6)	15.2 (5)

TTP: time-to-positivity, IQR: interquartile range, MIN: minimum, MAX: maximum, CoNS: coagulase-negative staphylococci, NFGNB: non-fermenting Gram-negative bacilli.

Gram-negative microorganisms

Concerning *Enterobacterales*, in 66.3% (590/890) the TTP was ≤ 12 hours. In the specific case of *E. coli*, 70.8% of the episodes became positive in ≤ 12 hours. Only 1.7% of the episodes produced by *Enterobacterales* required more than 48 hours to become positive, and only 0.9% yielded TTPs > 72 hours.

E. coli grew significantly faster than the rest of *Enterobacterales* ($P<0.0001$).

In the case of NFGNB, only one episode required less than 12 hours to become positive. Most of the episodes (66.7%) became positive in the 12-24-hour range. Only 4.2% of the episodes had a TTP greater than 48 h.

Enterobacterales grew significantly faster than NFGNB ($P<0.0001$).

According to our results, if the Gram stain of a positive BC that had flagged positive in less than or equal to 12 hours revealed Gram-negative bacilli, the probability of it being an *Enterobacterales* and not a NFGNB was 99.8% (95% CI, 98.9-99.9%).

Gram-positive microorganisms

In 36.1% of the episodes in which *S. aureus* was involved, flasks yielded positive in ≤ 12 h, and in 88.4%, the TTP was ≤ 24 h. Only 2.6% of the episodes became positive after 48 hours and in no episode the TTP was greater than 72 hours.

For CoNS, only 14.1% of the episodes grew in ≤ 12 h, and in 81.7% of the episodes the TTP was ≤ 24 h. Similar to what was stated for *S. aureus*, only 2.1% of the episodes became positive after 48 hours and in no episode the TTP was greater than 72 hours.

S. aureus grew significantly faster than CoNS ($P<0.0001$).

If the Gram stain revealed Gram-positive cocci in clusters of flasks that had signalled positive in less than or equal to 12 h, the probability of it being *S. aureus* and not a CoNS was 73.7% (95% CI, 62.1-82.8%).

For streptococci, 95.1% of the episodes grew in ≤ 24h, and, specifically, in the case of the episodes produced by *S. pneumoniae*, all of them became positive in less than 24 hours. In the case of enterococci, 93.3% of the episodes became positive in ≤ 24 hours.

While in more than half (61.2%) of the episodes caused by streptococci the TTP was ≤ 12 hours, in the case of enterococci, 32.6% of the episodes became positive in ≤ 12 hours and more than half (60.7%) became positive in the 12–24-hour range.

The TTP of *Streptococcus* spp. was significantly shorter than the TTP of *Enterococcus* spp. ($P<0.0001$). TTP of *S. pneumoniae* compared to other *Streptococcus* spp. did not show significant differences ($P=0.313$), nor did the TTP of *E. faecalis* and *E. faecium* ($P=0.079$).

Strict anaerobes

For strict anaerobes, TTPs were somewhat more heterogeneous. 36.8% of the episodes became positive in less than 24 hours, 40.4% in the 24-48-hour range and, 22.8% of the episodes required more than 48 hours to become positive.

Yeasts

In the case of *Candida* spp., 15.2% of the episodes took more than 72 hours to grow, but more than a half of the episodes (51.5%) grew in the 24-48-hour range. No significant differences were found in the TTP of *C. albicans* compared to other non-albicans *Candida* species (*P*=0.437).

For the two cases of *C. neoformans* isolated in 2019 in two HIV patients, the TTPs were 78.5 h and 117.6 h.

Bacteria harbouring resistance mechanisms

No significant differences were found in the TTP of ESBL or carbapenemase producing *Enterobacterales* compared to those that did not harbour these mechanisms (*P*=0.507).

Similarly, MDR *P. aeruginosa* did not grow significantly faster than the rest (*P*=0.553).

Likewise, no significant differences were found in the TTP of MRSA and MSSA (*P*=1.0).

The only episode produced by a microorganism harbouring transmissible resistance mechanisms with a TTP > 48 h, was an episode of urinary sepsis produced by an ESBL-producing *E. coli* isolate in a patient who had received antibiotic treatment in the 48 h prior to the extraction of BCs.

A.2. TTP analysis by source of infection

The predominant sources of BSIs were the urinary tract (32.6%), followed by the GI and biliary tract (23.8%) and the respiratory tract (12.4%). With respect to the source of infection, the shortest TTP was observed for episodes originating from the urinary tract and endocarditis.

Table 32 shows the TTP characteristics by source of infection.

Table 32. TTP by source of infection.

SITE OR TYPE OF INFECTION	n (%)	MEAN TTP (h)	MEDIAN TTP (IQR) (h)	MIN TTP (h)	MAX TTP (h)
Urinary tract	32.6 (582)	14.1	11.4 (10.1-13.4)	3.5	92.0
GI and biliary tract	23.8 (425)	17.6	11.6 (9.7-17.3)	2.9	98.0
Respiratory tract	12.4 (221)	16.0	12.2 (10.2-16.8)	2.9	120.0
Catheter related	10.2 (182)	18.0	15.5 (11.6-21.1)	5.2	98.0
Skin and soft tissue infection	5.1 (91)	17.0	13.6 (10.2-18.1)	6.2	88.0
Endocarditis	2.7 (48)	14.6	11.3 (9.1-16.6)	6.9	58.2
Bone and joint infection	1.4 (25)	22.9	15.4 (12.8-21.0)	6.6	159.0
Central nervous system	0.7 (12)	21.0	9.5 (7.7-16.4)	5.4	117.6
Other, multifactorial, and unknown	11.1 (198)	18.4	13.3 (10.1-19.4)	3.2	89.0

n: number, TTP: time-to-positivity, IQR: interquartile range, MIN: minimum, MAX: maximum, GI: gastrointestinal.

Significant differences were found in TTP according to the source of infection. As these differences could be due not so much to the source itself, but to the microorganism involved, only relevant foci or those in which the aetiologic agent is often similar were considered.

Figure 19 depicts the distribution of TTP by source of infection.

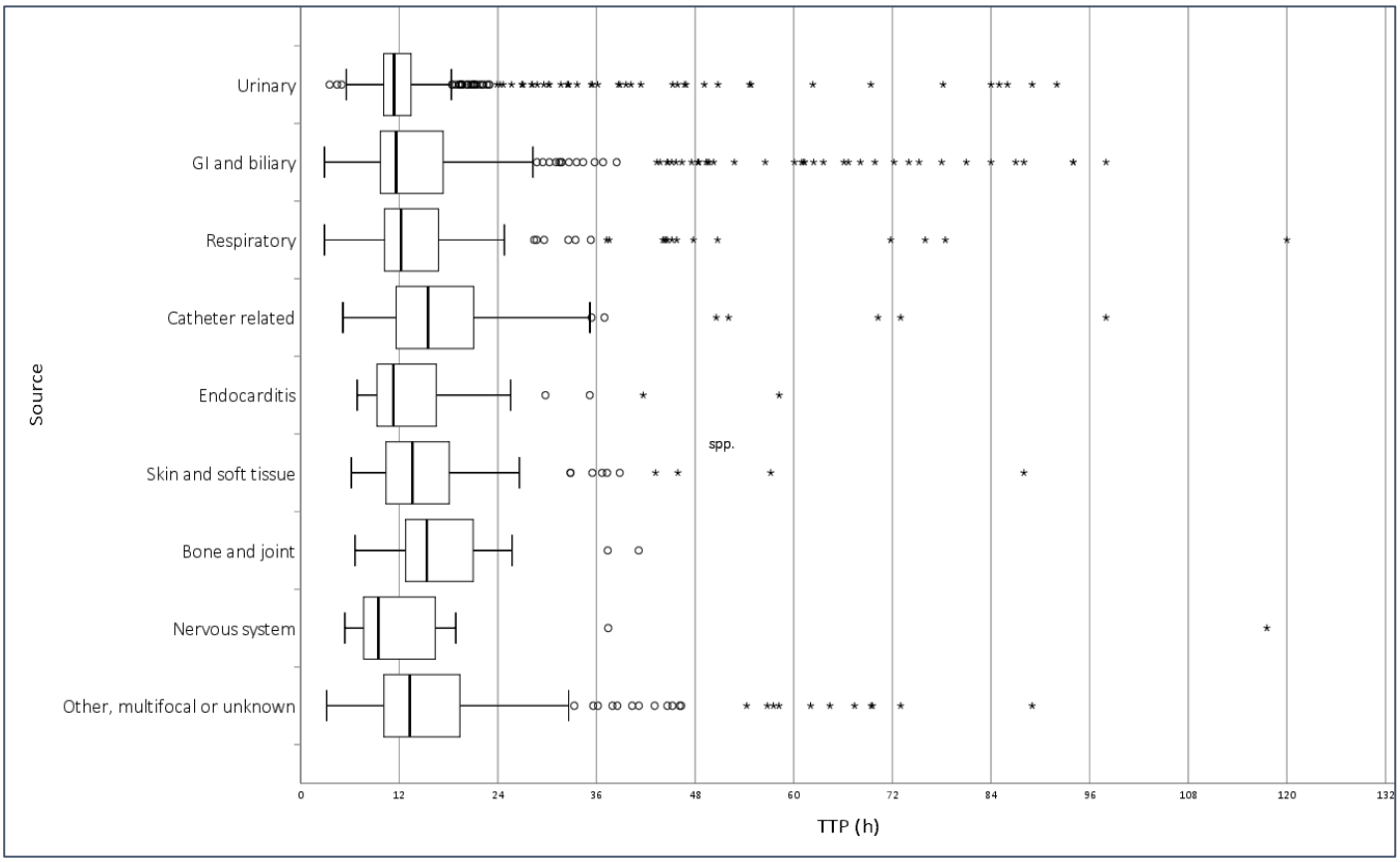


Figure 19. Box plot graph of the TTP by source of BSI. Circles represent outliers; asterisks represent extreme values. *TTP: time-to-positivity, GI: gastrointestinal.*

No significant differences were found in TTP belonging to patients with urinary tract infection and patients with GI and biliary infection (*P*=0.159); the same result was obtained when only *Enterobacterales* were evaluated (*P*=0.076).

The TTP of episodes in which *S. aureus* was involved was significantly lower in infective endocarditis than in the rest of infections (*P*=0.011). This difference was not noted in the cases of CoNS (*P*=0.335) and of *E. faecalis* (*P*=0.212).

A.3. BSI characteristics of episodes with TTP > 24 h and TTP > 48 h

A total of 226 episodes became positive after 24 hours. The characteristics of these episodes are summarized in Table 33.

Table 33. Characteristics of episodes with TTP greater than 24 h.

TTP (h)	Age ($\bar{X}$)	Sex	Petitioner	Aetiology	Source of BSI
24-48 (158)	67	65.2% male	47.5% ED 17.7% ICU	1. *Enterobacterales*: 16.9% 2. CoNS: 14.6% 3. Strict anaerobes: 14.6% 4. Yeasts: 10.8% 5. *Pseudomonas* spp.: 9.5% Polymicrobial: 3.8%	1. GI and biliary tract: 27.2% 2. Urinary tract: 18.4% 3. Catheter related: 17.1% 4. Unknown: 11.4% 5. Respiratory tract: 11.4%
> 48 (68)	66	75.0% male	58.8% ED 13.2% ICU	1. *Enterobacterales*: 23.5% 2. Strict anaerobes: 19.1% 3. Yeasts: 19.1% 4. *Campylobacter* spp.: 10.3% 5. *S. aureus*: 5.9% Polymicrobial: 4.4%	1. GI and biliary tract: 41.1% 2. Urinary tract: 17.6% 3. Unknown: 10.3% 4. Catheter related: 7.4 % 5. Respiratory tract: 7.4%

TTP: time-to-positivity, BSI: bloodstream infection, ED: Emergency Department, ICU: Intensive care unit, CoNS: coagulase-negative staphylococci, GI: gastrointestinal.

In 8.9% of the episodes (158/1784) the TTP was in the 24-48-hour range; 32.9% of these episodes were caused by strict anaerobes (14.6%), yeasts (10.8%), and rarer microorganism. Excluding these that, per se, are slow growing, in the remaining episodes,

35.8% the patient had received prior antibiotic treatment in the 48 h prior to the extraction of BCs.

In 3.8% of the episodes (68/1784) TTP was > 48 h. Slightly more than half of these episodes were caused by yeasts (52.9%), strict anaerobes (19.1%), *Campylobacter* spp. (10.3%), and other rare microorganisms (4.4%). Excluding these, in 37.5% of the remaining episodes, the patient had received antibiotic treatment before the extraction of BCs.

Overall, the 28-day mortality in episodes that grew > 24 h was of 17.7%.

A.4. Candidaemia episodes

In a large prospective multicenter randomized control trial published in 2021 (99), the relationship between TTP and mortality was evaluated, finding that TTP was not associated with mortality except in the case of *Candida* spp. (longer times associated with worse outcomes) and possibly *Streptococcus* spp.. Therefore, it was decided to examine the 28-day mortality only for the episodes of candidaemia.

The thirty-three episodes produced by *Candida* spp. were further analyzed. Fifteen were identified as C*. albicans*, seven as *C. glabrata*, four as *C. parapsilosis*, and the remaining seven belonged to different species. Mean age was 72 years, 54.5% of the episodes occurred in men.

BCs were ordered primarily in the ICU (42.4%), the ED (18.2%), and the Surgery Department (15.2%).

The fungaemia episode with the lowest TTP (13.2 h) occurred in an oncological patient admitted to the ICU with bacteraemia of intestinal origin. The highest TTP was 120 h in a patient who had received appropriate antifungal treatment prior to the extraction of BCs. This episode was excluded from the 28-day mortality analysis.

In all, fourteen (43.8%) patients died. A relationship between TTP mortality was not found.

B. Contaminated episodes

A total of 1106 episodes considered contaminations was included, 518 episodes were recovered in 2019, and 588 in 2020.

The TTP characteristics of episodes considered contaminations are depicted in Table 34.

Table 34. TTP by type of microorganism of contaminated episodes.

	n (%)	MEAN TTP (h)	TTP MEDIAN (IQR) h	MIN TTP (h)	MAX TTP (h)
Total	1106	29.3	22.6 (18.9-31.5)	8.8	118.4
Monomicrobial	983	30.5	23.2 (19.4-33.0)	9.3	118.4
Mixed contamination	123	19.5	18.1 (15.6-22.1)	8.8	46.4
CoNS (monomicrobial)	841	26.5	25.6 (19.2-29.4)	10.7	113.0
CoNS (monomicrobial and mixed)	933	25.8	22.2 (19.0-28.5)	10.1	113.0
Streptococcus spp.	28	18.4	15.9 (12.3-22.3)	9.3	41.6
C. acnes	26	102	113.3 (101.5-113.3)	72.2	118.0
Corynebacterium spp.	25	59.8	46.4 (35.7-83.0)	21.1	118.4
M. luteus	13	47.5	42.6 (31.8-64.5)	26.3	74.0

n: number, TTP: time-to-positivity, IQR: interquartile range, MIN: minimum, MAX: maximum, CoNS: coagulase-negative staphylococci.

In the contamination group, median TTP was 22.6 (IQR, 18.9-31.5) h, and was significantly higher than the one for true BSI (*P*<0.0001).

As with the real episodes, polymicrobial contaminated episodes grew significantly faster (*P*<0.0001) than the monomicrobial ones.

No correlation was found between age and TTP.

CoNS accounted for 84.4% (933/1106) of the contaminated episodes, followed by *Streptococcus* spp., *C. acnes, Corynebacterium* spp. and *M. luteus*. Figure 20 depicts the distribution of TTP by type of microorganism considered contamination.

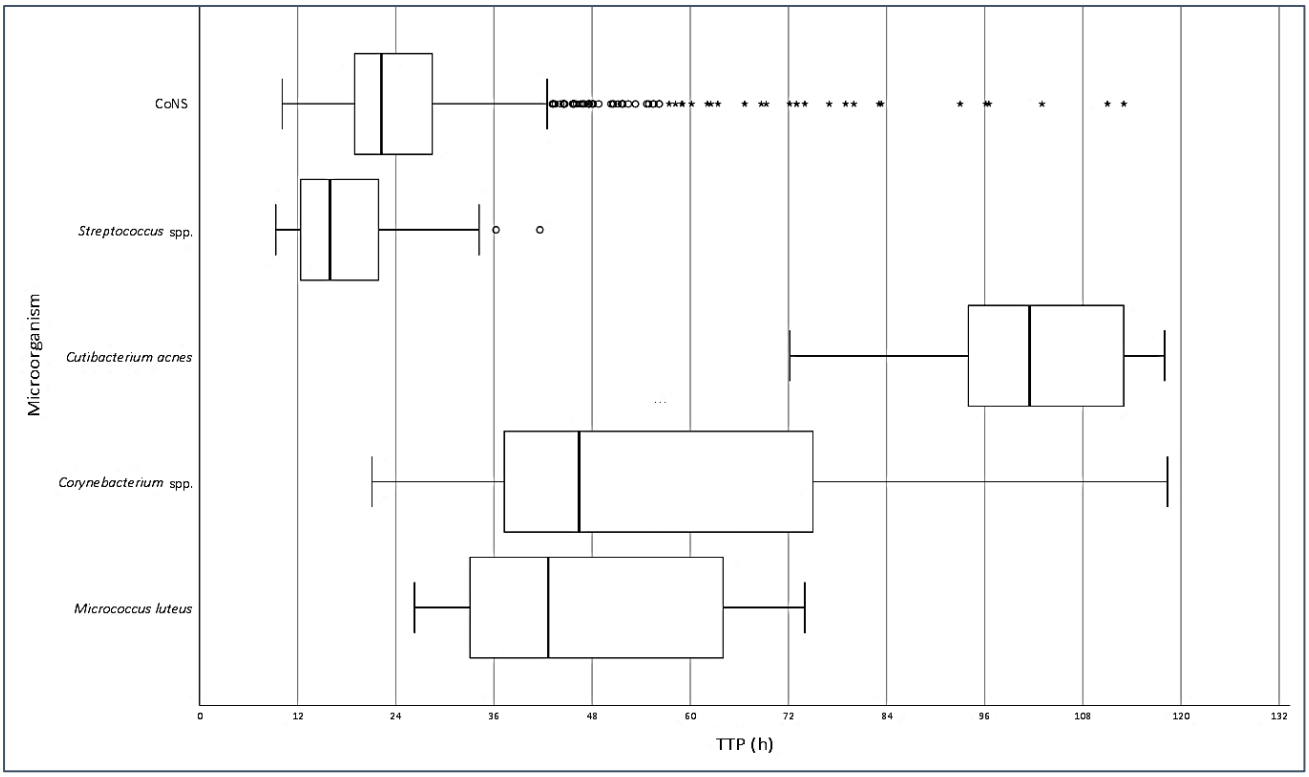


Figure 20. Box plot graph of the TTP by type of microorganism of contaminated episodes. Circles represent outliers; asterisks represent extreme values. *CoNS: coagulase-negative staphylococci, TTP: time-to-positivity.*

When a flask signalled positive after 24 h, the probability of contamination was 68.0% (95% CI, 64.4-71.4%); excluding yeasts and rare microorganisms the probability was 73.4% (95% CI, 69.8-76.8%). Considering only CoNS, the probability of contamination after 24 h was 93.3 % (95% CI, 90.3-95.6%).

The most isolated contaminant was *S. epidermidis* (458 episodes [41.4%]), followed by *S. hominis* (397 episodes [35.9%]).

Only one *S. aureus* episode was considered a contamination.

Regarding CoNS, bottles of contaminated episodes yielded TTP significantly higher (P<0.0001) than the ones of real BSI, and, specifically, flasks of true *S. epidermidis* episodes flagged positive considerably faster (P<0.0001) than the ones considered contaminations. Likewise, bottles belonging to true BSI episodes in which *S. hominis* was involved grew significantly faster (P=0.018) than in those considered contaminations. Nevertheless, when children's CoNS episodes were evaluated separately, no significant differences were found in the TTP of real BSI and contamination (P=0.152).

Streptococcus spp. involved in real BSI episodes yielded TTPs significantly lower (P<0.0001) than those considered contaminations.

B.1. PBDP and rates of true BSI and contamination

The PBDP of all CoNS episodes was evaluated, excluding episodes that occurred in paediatric patients.

Figure 21 A. depicts the distribution of PBDP and TTP of all CoNS episodes by type of BSI and Figure 21 B. depicts separately the distribution of PBDP and TTP of *S. epidermidis* and *S. hominis* episodes by type of BSI.

In the real BSI group, the most frequent pattern was Pattern 5 (61.6%); Patterns 5 and 4 accounted for 79.0% of the real episodes and only 7.2% of the episodes featured Pattern 1 (0.7%) and Pattern 2 (6.5%). None of the episodes considered real was produced by two or more different species of CoNS.

In the contamination group, 96.1% of the episodes considered contaminations yielded Patterns 1 (83.4%) and 2 (13.5%); considering mixed contaminated CoNS episodes, 93.9% yielded Patterns 1 (75.9%) and 2 (18.1%). Patterns 4 and 5 occurred very infrequently, 0.6% in the case of episodes in which a single species was implicated, and 2.0% in which two or more CoNS species were implicated.

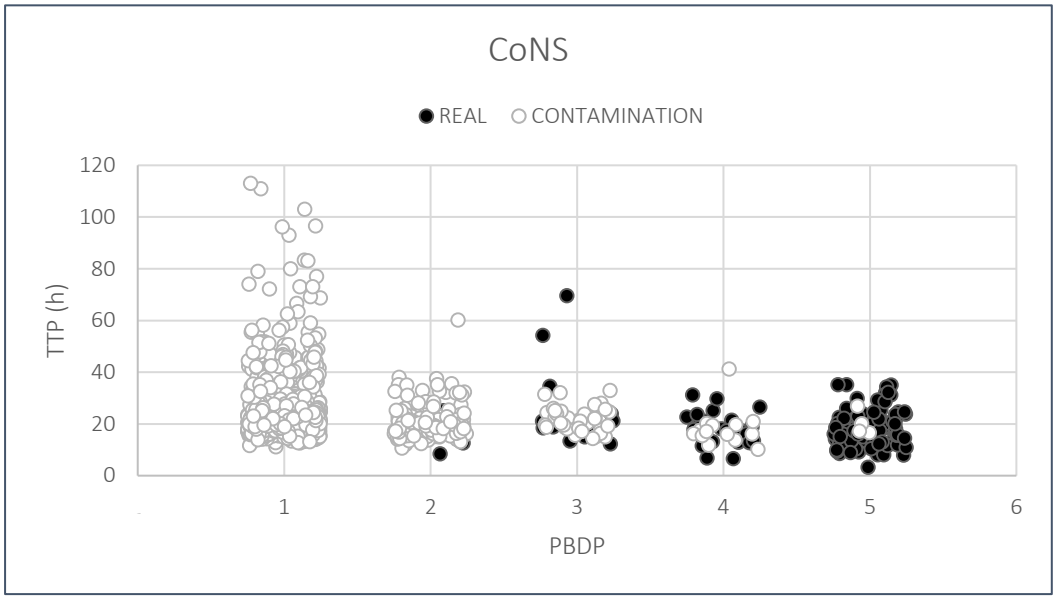


Figure 21 A. Distribution of PBDP and TTP of all CoNS episodes by type of BSI. *CoNS: coagulase-negative staphylococci, TTP: time-to-positivity, PBDP: positive bottle detection pattern.*

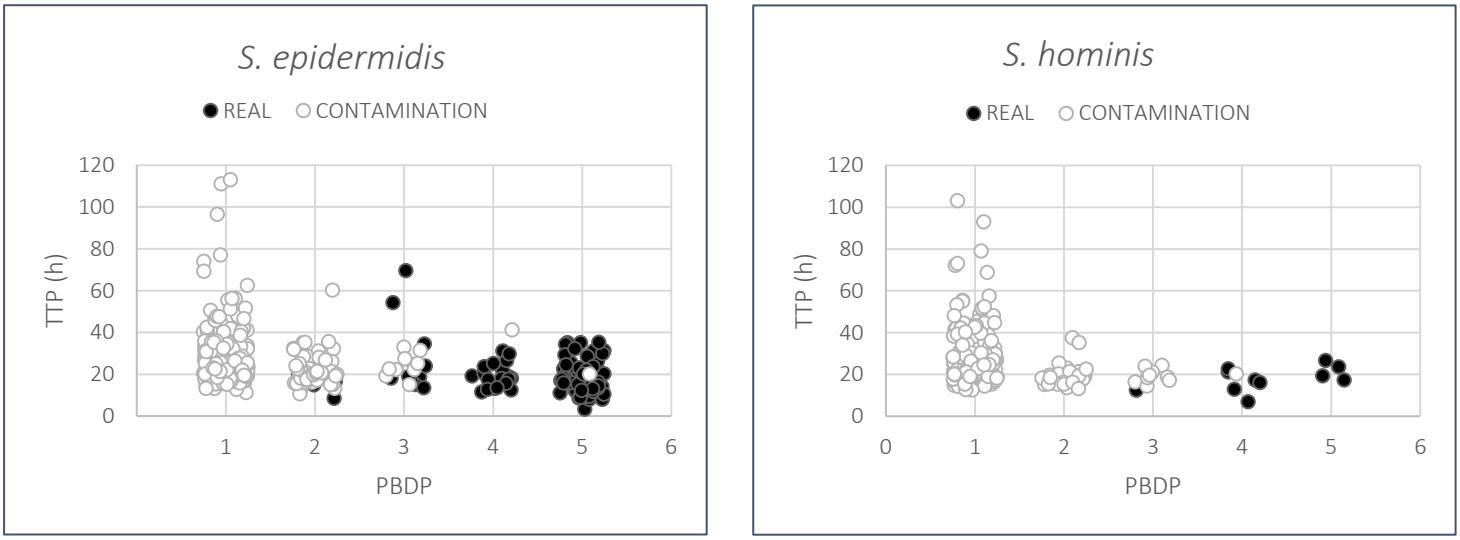


Figure 21 B. Distribution of PBDP and TTP of *S. epidermidis* and *S. hominis* episodes by type of BSI. *TTP: time-to-positivity, PBDP: positive bottle detection pattern.*

BLR for prediction of BCC by CoNS

BLR analysis was used to examine the influence of TTP and PBDP on BCC by CoNS, since both variables are significantly associated with BCC.

The result of the logistic regression is presented in Table 35.

Table 35. BLR model results.

	B	*SE*	Wald	gl	Sig.	Exp (B)
TTP	0.002	0.023	0.007	1	0.932	1.002
PBDP (Pattern 1 ref. cat)			144.318	4	0.000	
Pattern 2	-3.619	1.069	11.469	1	0.001	0.027
Pattern 3	-5.582	1.054	28.068	1	0.000	0.004
Pattern 4	-7.210	1.073	45.168	1	0.000	0.001
Pattern 5	-9.610	1.139	71.191	1	0.000	0.000
Constant	6.482	1.177	30.334	1	0.000	653.113

TTP: time-to-positivity, PBDP: positive bottle detection pattern.

Accordingly, the following formula was inferred to determine the association between TTP and PBDP in BCC.

$$p = \frac{e^{6,482+0,002[time]-3,619[D1]-5,582[D2]-7,21[D3]-9,61[D4]}}{1+e^{6,482+0,002[time]-3,619[D1]-5,582[D2]-7,21[D3]-9,61[D4]}}$$

This model was fitted in Excel and gave an interactive table that allows seeing how each variable affects in BCC and determining the probability of contamination. Thus, to provide an example, when a bottle has a TTP of 24 h and a Pattern 1 the probability of contamination is 99.9%, when the PBDP is 2, the probability is 94.8%, with a Pattern 3 it

is of 72.1%, if it is 4 it is 33.6% and, finally, if the Pattern is 5 the probability of contamination falls to 4.4%.

OBJECTIVE 4

Impact of the SARS-CoV-2 pandemic on blood culture utilization and characteristics of bacteraemia in SARS-CoV-2 patients

On February 28, 2020, the first case of SARS-CoV-2 was microbiologically diagnosed in our laboratory. The rapid spread of this virus across our area led to a surge of febrile patients presenting to our institution.

This brief analysis aims to assess the impact of the pandemic in BC utilization and BSI among COVID-19 (Coronavirus disease 2019) patients.

The number of BCs/1000 admissions ordered in 2020 statistically increased (*P*=0.018) in 5.1% (95% CI, 1.5-9.9; *P*=0.011) compared to 2019, with a marked peak registered in March (27.7 per 1000 admissions in 2019, compared to 50.3/1000 admissions in 2020).

Excluding doubtful or inconclusive episodes, and as stated in the previous section, the number of real episodes recovered was similar in both years.

BCC increased in 2020, while in 2019 36.7% of the episodes were considered contaminated episodes, in 2020 were 39.8%. However, this increase was not statistically significant (*P*=0.092)

Figure 22 depicts the distribution of episodes considered contamination per month and year.

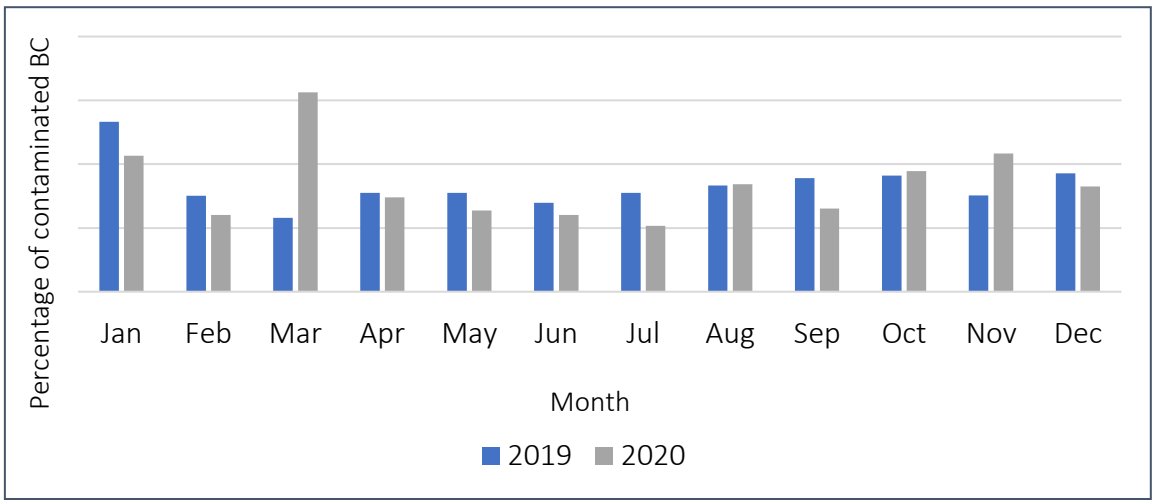


Figure 22. Distribution of episodes considered contamination per month and year. *BC: blood culture*.

The incidence of BSI caused by ESKAPEEc remained practically unchanged (~60%). Likewise, candidaemia rates did not statistically increase.

As mentioned in Objective 1, a pronounced decrease in *S. pneumoniae* bacteraemia was experienced (P<0.001).

Linezolid resistance rates significantly increased from 8.9 to 25% (P=0.003). Nevertheless, BSI caused by ESBL producing *Enterobacterales*, MDR *P. aeruginosa,* and MRSA did not significantly increase.

From 28 February to 31 December, 729 real BSI episodes (excluding doubtful episodes) were recorded, of which 10.3% (75/729) belonged to SARS-CoV-2-positive patients (confirmed by RT-PCR).

The most common aetiology, source, and site of acquisition of BSI, as well as the requesting department are summarized in Table 36.

Table 36. Characteristics of BSIs in COVID-19-positive and COVID-19-negative patients.

SARS-CoV-2	AGE ($\bar{X}$)	SEX	AETIOLOGY	MICROBIOLOGICAL SOURCE	CO and HO	PETITIONER
POSITIVE	66	78.7% male	1. *S. epidermidis* (20.0%) 2. *Enterococcus* spp. (14.7%) 3. *S. aureus* (12.0%) 4. *E. coli* (10.7%) 5. *Candida* spp. (9.3%)	1. Catheter related (37.3%) 2. Respiratory tract (28.0%) 3. Unknown (25.3%)	CO: 10.7% HO: 82.7%	ED: 10.7% ICU: 89.3%
NEGATIVE	71	65.7% male	1. *E. coli* (39.9%) 2. *S. aureus* (9.6%) 3. *K. pneumoniae* (7.3%) 4. *Enterococcus* spp. (6.9%) 5. *S. epidermidis* (5.0%)	1. Urinary tract (36.2%) 2. GI and biliary (26.9%) 3. Unknown (11.0%)	CO: 53.5% HO: 22.7%	ED: 69.7% ICU: 5.0%

SARS-CoV-2: severe acute respiratory syndrome coronavirus-2, CO: community-onset, HO: hospital-onset, GI: gastrointestinal, ED: Emergency Department, ICU: Intensive care unit.

Among the 75 SARS-CoV-2-patients, mean age was 66 years and 78.7% were men. The mean age was significantly lower among COVID-19 patients than in the rest (P=0.018). 89.3% (67/75) of the BSI were either HO or HCA. 73.3% belonged to BC ordered in the ICU. The mean hospital stay was 20 days. For COVID-19 negative patients, just over half of the episodes were CO and the predominant requesting department was the ED.

While among SARS-CoV-2-patients the main microorganisms involved were CoNS (21.3%), *Enterobacterales* (20.0%, *E. coli* 10.7%), *Enterococcus* spp. (14.7%), *S. aureus* (12.0%), NFGNB (10.7%), and *Candida* spp. (9.3%) (Figure 23), among non-COVID-19 patients *E. coli* and *S. aureus* were the leading causes of BSI and only 5.0% of the episodes were caused by *S. epidermidis*.

The main source of BSI was catheter-related bacteraemia (37.3%) followed by the respiratory tract (28.0%).

Both the spectrum of microorganisms involved, and the source of infection were those expected for patients with long hospitalizations.

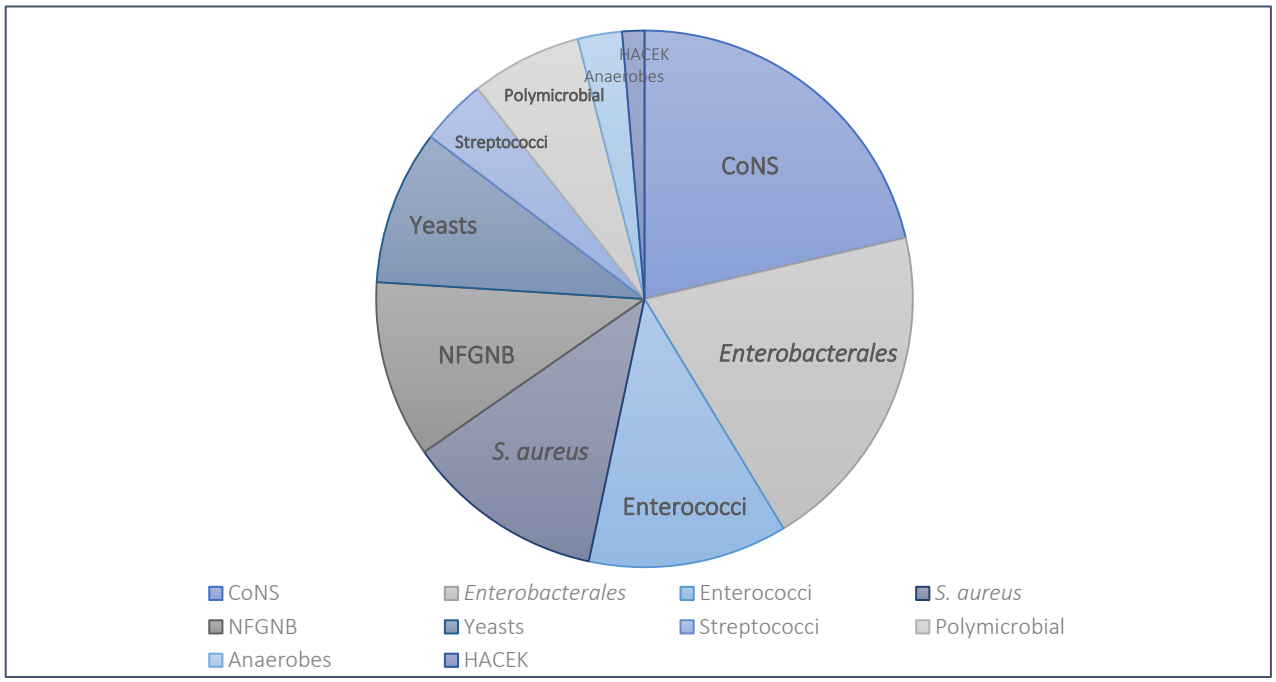


Figure 23. Spectrum of microorganisms involved in BSI in SARS-CoV-2-patients. *CoNS: coagulase-negative staphylococci, NFGNB: non-fermenting Gram-negative bacilli, HACEK: Haemophilus* spp.*, Aggregatibacter* spp.*, Cardiobacterium* spp.*, Eikenella* spp. *and Kingella* spp..

DISCUSSION

Sepsis is an extremely serious clinical syndrome, with a very high mortality rate, an increasing incidence, and important associated costs. All this has generated the need to optimize both its diagnosis, at a clinical and microbiological level, as well as its management. This work involves professionals from different fields: clinicians, microbiologists, pharmacists, and preventive medicine physicians, mainly.

Time is the most important variable that influences mortality and costs derived from sepsis. In the study by Kumar et al., it was demonstrated that the administration of an effective antimicrobial for the isolated or suspected pathogens within the first hour of documented hypotension was associated with a survival rate of 79.9%. Likewise, each hour of delay in the administration of antimicrobials for the next six hours was associated with an average decrease in survival of 7.6% (100). On the other hand, it is estimated that 40-50% of adult patients with bacteraemia (and 70% with fungaemia) received incorrect antimicrobial therapy during their empirical treatment period before microbiological culture results were available (101). Therefore, rapid and accurate microbiological diagnosis of the causative microorganism and its susceptibility profile is required.

BCs continue to be the cornerstone of sepsis diagnosis, as it is a prerequisite for AST. For this reason, the Microbiology Department plays a fundamental role in the BSI and sepsis diagnosis. Optimizing existing diagnostic techniques reduces response time. Nevertheless, although the advancement in new technologies including automation, MALDI-TOF or newly launched molecular platforms enables a dramatic speed up in

microbiological diagnosis, without a 24/7 operating Microbiology Laboratory and a microbiologist on call, these improvements are completely ineffective.

This work focuses on the study of culture-based diagnosis of bacteraemia, analysing the epidemiology, aetiology, and resistance profiles to key antibiotics of the main microorganisms responsible of BSI isolated in a core Microbiology Department during a six-year period, evaluating two RAST assays and investigating the potential role of TTP in the diagnosis of bacteraemia. Likewise, since the pandemic broke out during the study period, the impact of the SARS-CoV-2 pandemic on BC utilization is evaluated, as well as the characteristics of bacteraemia in patients with SARS-CoV-2.

OBJECTIVE 1

Bloodstream infections: incidence and trends at a tertiary and a district hospital during a six-year period (2015-2020)

Here is provided an overview of the epidemiology, aetiology, and resistance profiles to key antibiotics of the main microorganisms responsible of BSI isolated in a core laboratory during a six-year period, including a SARS-CoV-2 pandemic period (February 2020-December 2020).

During the study period, no significant increase in the BC workload was experienced. The number of BCs/1000 admissions ordered in 2020 statistically increased in 5.1% (95% CI, 1.5-9.9; *P*=0.011) compared to 2019, with a marked peak registered in March coinciding with the advent of the SARS-CoV-2 pandemic. This issue will be addressed in the Discussion section regarding Objective 4.

Currently published guidelines do not clearly state when BCs should be requested. As mentioned in the Introduction section of this document, BC are routinely ordered when the patient experiences signs and symptoms of sepsis, leucocytosis, fever of unknown origin, suspected endocarditis or prior to starting antimicrobial treatment in elderly or immunocompromised patients, among others. In a study carried out by Poses et al. it was demonstrated that physicians significantly overestimated the likelihood of bacteraemia

for most of their patients (102). In fact, in most settings, only 5 to 13% of BCs will test positive, and of these, 20 to 56% represent contaminants (103).

BSI episodes/1000 admissions in our tertiary hospital significantly increased during the study period. Nevertheless, no upward trend was observed in our district hospital.

In children the incidence of bacteraemia episodes per 1000 admissions remained stable.

The incidence of polymicrobial episodes significantly increased in HUA, not so in HAD. bacteraemia rate was significantly higher in the tertiary hospital than in the district hospital. Several studies have reported a higher mortality rate in patients with polymicrobial bacteraemia than those with monomicrobial infection (104,105), although Sancho et al. reported that polymicrobial BSI did not have any influence on the related mortality, but they only evaluated ICU patients (106). Polymicrobial BSIs have been associated with higher Acute Physiology and Chronic Health Evaluation (APACHE) II scores and, expectedly, were more likely to express as severe sepsis and/or septic shock compared with patients with monomicrobial bacteraemia (105). Besides, in patients with polymicrobial BSI the length of stay and the length of stay post-infection have been reported to be significantly longer (106). The higher rates of polymicrobial BSIs found in the tertiary hospital compared to the district one may be due to the fact that in the former there are critical, oncological, or palliative patient units, that is, more seriously ill patients are cared for. In the study period, no polymicrobial BSI episode was obtained nor in children nor in neonates, although polymicrobial bacteraemia has been reported to be relatively common among neonates comprising nearly 14% of all infectious episodes

(107). In this regard, it should be mentioned that seriously ill neonates are referred to another specialized hospital (Hospital Universitario of Cruces, Barakaldo).

BSIs caused by Gram-negative and Gram-positive bacteria and by yeasts increased in one or another hospital, while strict anaerobic BSIs remained uniformly stable.

Gram-negative bacteria remained the predominant cause of BSI and a significant increase in Gram-negative BSI incidence was observed in both hospitals during the study period. It has been estimated that Gram-negative bacilli are the cause of approximately a quarter to half of all BSIs (108). In a large study evaluating trends in the aetiology of BSIs over a 22-year period (1985-2006) in Spain, Gram-positive pathogens were found to have outperformed other aetiologic agents of BSIs (109). Nevertheless, in more recent studies a shift toward predominance of Gram-negative BSI has been described (110). In any case, empirical treatments for BSI should be guided and adapted to local epidemiology.

Our tertiary hospital experienced an increase in the incidence of BSIs caused by CoNS, which is likely justified by the presence of departments such as haematology, oncology, or ICU, and is probably due to the massive use of intravascular catheters and other devices. Progress in medicine and improvement in medical management and life expectancy of patients leads to an increased number of immunocompromised patients. In these, the use of medical devices along with their underlying disease and, in some cases, long-term hospitalizations, leads to an increase of infections caused by opportunistic microorganisms such as CoNS (111).

Like other reports (112), in 2020 and coinciding with the advent of the SARS-CoV-2 pandemic, our settings experienced a marked decline in invasive pneumococcal disease which is likely attributable to public health measures, such as social distancing, mask wearing, and limiting the size of group gatherings.

In the study, the incidence of obligate anaerobic BSI remained stable, and no episode was isolated in children. Although some authors have suggested that anaerobic BCs should only be used selectively, whenever clinical signs and symptoms are suggestive of anaerobic BSI (113), in our hospitals, anaerobic cultures are routinely performed in adult patients. Nevertheless, we do not offer routine anaerobic BCs in children. Recent studies support the inclusion of anaerobic BCs in these patients despite the low recovery rate of obligate anaerobes based on the increased recovery of facultative anaerobes from anaerobic BCs (114).

Our data show an increase in the incidence of candidaemia in our district hospital, which is in accordance with previous studies from different geographical areas. The overall candidaemia incidence/1000 admissions was 0.4 and 0.2 in HUA and HAD; our data reveal a lower incidence than that reported in Greece (range, 1.1-1.9) (115), and was comparable to that reported in Norway (range, 0.2–0.3 (116)). Although *C. albicans* is still considered the main causative *Candida* species, a shift to non-albicans *Candida* species is globally observed (115,117). In our study, a little more than a half (55.7%) of the candidaemia episodes were caused by non-albicans *Candida* spp. The epidemiology of *Candida* infections has been reported to be changing in the last decade, with a gradual shift from *C. albicans* to non-albicans candida strains which may be less susceptible to

azoles (118). Differences in *Candida* epidemiology have been related to several contributing factors such as haematological malignancy, or the use of certain antimicrobials and azoles (119). At all events, these changes might vary between hospitals and regions depending on the type of risk factors in the population and antifungal used.

Overall, 62.9% of the BSIs belonged to BCs ordered in the ED. Among patients with BSI, the administration of inadequate empirical treatments is independently associated with an increase in mortality (4). Empirical antimicrobial treatments and regimens are chosen based on clinical and local epidemiological data and antibiotic guidelines should be adapted, reviewed, and updated periodically. To that end, monitoring resistance levels of key antimicrobials such as third-generation cephalosporins, carbapenems and vancomycin using surveillance systems is of great importance. Knowledge of the aetiology and resistance patterns of BSIs belonging to the ED is of particular significance since antimicrobial treatment is frequently initiated here.

In line with the above, most Antimicrobial Stewardship Programs (ASPs) are primarily focused on inpatient management although the EDs are the entrance gates for patients presenting with infectious diseases into the hospital and a significant part of hospital antibiotic prescribing is initiated here (120). In the ED setting, antimicrobial treatment is typically empiric and clinical decision making must occur fast, and, in most cases, in the absence of microbiological results or feedback regarding patient course and outcomes. It is estimated that around half of the prescriptions made in the EDs are either unnecessary or inappropriate, making the ED a crucial focus of ASPs (121). This rate is unacceptably

high if we consider that full adherence to guidelines for all aspects of antimicrobial prescribing is associated with a reduction in mortality (122).

The ESBL phenotype was observed in 8.7% (244/2801) of the *Enterobacterales* isolated and the incidence did not fluctuate over the years. Likewise, 0.7% (21/2801) of the *Enterobacterales* yielded elevated ertapenem MICs (> 0.5 mg/L), but carbapenemase production could only be demonstrated in 0.2% (6/2801); no trend was observed. Our data reveal that the rate of ESBL and carbapenemase production has remained stable in the last six years. In a similar way, the study conducted by Le Page et al. demonstrated a global low level of resistance to key antibiotics in Marseille (France) when evaluating retrospectively antibiotic susceptibility data from 2001 to 2016 (123). However, and as the authors of the aforementioned article imply, active epidemiological surveillance is demanded because antibiotic resistance remains unpredictable.

Regarding *P. aeruginosa*, 8.8% of the isolates presented the MDR phenotype. MDR *P. aeruginosa* has remained stable in the last six years. Although the terms MDR, extensively-drug resistant (XDR), and pandrug-resistant (PDR) are widely used there is still some degree of disparity in the definitions used, as precise definitions are lacking as in the case of *Mycobacterium tuberculosis*. The traditional MDR definition for Gram-negative bacteria (acquired non-susceptibility to at least one agent in three or more antimicrobial categories) is a consensus definition provided by the European Center for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC), but it is difficult to apply and often ignores new antimicrobials and large panels of antibiotics (123). The definition proposed by the German Society for Hygiene

and Microbiology (97), which is the one followed along this document, is based on evaluation of the four groups of antibiotics that have bactericidal effects: penicillins, cephalosporins, carbapenems, and fluoroquinolones. Aminoglycosides are not included, as they should only be used in combination therapy, nor are bacteriostatic antibiotics. Thus, a microorganism is MDR when only surrogate substances representing no more than one of these groups of antibiotics still yield a test result indicating susceptibility. In the present work and in daily clinical practice we use this definition as it is easier and more practical to follow.

The MRSA rate did not increase over the years and only one MRSA isolate displayed vancomycin and teicoplanin MICs of above 2 mg/L. In the annual epidemiological report for 2019, the European Antimicrobial Resistance Surveillance Network (EARS-Net) (124) reported a decline in the percentage of MRSA isolates, although this trend was not observed in our study.

The emergence of MRSA has been the most notable problem in the evolution of resistance in Gram-positive bacteria, but the massive use of linezolid, especially in the ICU, is leading to a worrying increase in isolates resistant to this compound (125). In fact, linezolid-resistant staphylococci strains have been reported increasingly all over the world, due, probably to its unregulated use (126–129). Optimizing antibiotic use and implementing infection prevention and control interventions may hamper the spread and frequency of infections due to such bacteria. Awareness amongst clinicians should be raised about the importance of judicious and relevant use of antibiotics. To comprehensively understand the current resistance mechanisms among linezolid-

resistant clinical isolates belonging to BSI episodes, strains isolated during the last two years of the study (2019-2020) have been collected and are going to be further analysed.

For BFG, resistance rates reported in this study are comparable to those published in previous studies (130). An increase in the rate of resistance to amoxicillin-clavulanate was observed during the study period, which may be due to the widespread use of this antibiotic in our area. This trend was already observed in another Spanish study (131). Since changing patterns of susceptibility to BFG strains isolated in our hospital have been observed, monitoring these patterns is extremely important to guide the selection of appropriate antimicrobial therapy.

Routine antifungal susceptibility testing of all BSI *Candida* species is common practice in our Laboratory. Antifungal resistance levels were low in our study which is in accordance with other published data (132), making it necessary for any finding of high levels of resistance to be verified. Despite the low antifungal resistance rates observed, it is necessary to continue monitoring the epidemiology and resistance profiles to detect possible significant shifts.

Since susceptibility breakpoints defined by the main committees are subject to constant changes over the years, resistance trends should be performed, if possible, based on MICs and not on qualitative criteria (susceptible or resistance). Commercial panels can hinder studies evaluating susceptibilities over several years because, often, they do not allow knowing the exact MIC but that it is below a breakpoint that can change over the years.

As mentioned before, in addition to emphasizing the value of early empirical therapy, it is important to ensure that BSIs are diagnosed accurately and that infecting pathogens, their antimicrobial susceptibilities, and the possible primary sources of infection are evaluated thoroughly, to enable optimal targeted antimicrobial therapy. Therefore, rapid, and accurate microbiological diagnosis of the causative microorganism and its susceptibility profile is required, as well as a 24/7 operating Microbiology Laboratory and an on-call microbiologist available to report results. In this line, although ASPs for optimizing antibiotic use and minimizing adverse events are being widely implemented with positive results, the high workload, and the scarce human resources limit, to a large extent, the optimal functioning of these programs.

Study limitations

The study has some limitations:

1. The study was retrospective and only two hospitals were involved.
2. Clinical aspects such as the source of infection, severity, or outcome were not evaluated.
3. BSIs could not be classified in CO and HO. However, this classification is often artificial and overlooks the complex role community medical care (e.g., nursing homes, haemodialysis unit, specialized in-home medical services) plays in infection. Nevertheless, we were able to analyse BSI episodes of BCs ordered in the ED.
4. The study may have overestimated the incidence of CoNS since we included all those episodes that were initially considered significant and to which an AST was

performed. However, even following both clinical and microbiological criteria, the interpretation of positive BCs for CoNS is often troublesome (133). Studies based on retrospective data export may oversize the number of episodes because in doubtful or inconclusive cases, an antibiogram is usually performed and consequently and/or implicitly the episode is given microbiological significance. For this reason, in this type of study, when interpreting data, this fact must be considered especially in the case of microorganisms whose presence is not always indicative of infection, as in the case of CoNS.

5. Some resistance mechanisms may have been overlooked.

OBJECTIVE 2

Evaluation of two assays using E-tests and VITEK®-2 for rapid detection and accurate differentiation of bacterial antibiotic susceptibility in *Enterobacterales*

This study evaluated two assays using E-tests and the Vitek®-2 for rapid detection and accurate differentiation of bacterial antibiotic susceptibility in *Enterobacterales*.

As mentioned above and as a note of clarification, it should be noted that the study was designed and conducted before EUCAST published its guidance on RAST.

Both protocols used are easy to implement, inexpensive (especially when compared with new platforms that provide antibiograms such as Accelerate Pheno® system [Accelerate® Diagnostics]), do not require special equipment or complicated sample handling.

Both methods allow reading MICs after 5 hours, although the Vitek®-2 method does not ensure that MICs for all antibiotics are available after that time. In fact, while for amoxicillin-clavulanate and for ciprofloxacin MICs were available after 5 h in > 95% of the isolates analysed, for imipenem and piperacillin-tazobactam only less than half and less than 10% of the strains had MICs available after 7 hours, respectively. Nevertheless, one of the advantages of the Vitek®-2 assay over the E-test is that that in it MICs do not vary over the time.

Comparative evaluations of susceptibility testing systems have suggested that VME should occur in < 1.5% of all tests, and the overall agreement between test and reference method should be superior to 95% (134). In our study the VME rate was 0.8% for the E-test method (for both readings), while for the Vitek-2 method the VME rate was 3.3% in the case of gentamicin, 1.7 % for amikacin and 0.8% for ciprofloxacin. Total agreement for all antibiotics was > 95%, except for amoxicillin-clavulanate, piperacillin-tazobactam and ciprofloxacin in the case of the Vitek®-2 method, for amoxicillin-clavulanate, piperacillin-tazobactam, ciprofloxacin and amikacin in the case of the E-test-based method with readings after five hours of incubation. However, for the E-test-method with readings after seven hours, an agreement of more than 95% was obtained except for amoxicillin-clavulanate and amikacin.

The results of this study demonstrate the usefulness of a direct AST method from positive BCs that allows same-day antimicrobial susceptibility assessment of *Enterobacterales*. Unlike disk diffusion, both assays evaluated allow easy determination of MIC values that are increasingly relied upon for antimicrobial administration, especially with some drugs and bacteria. Nevertheless, the results obtained were suboptimal for both proposed methods, especially in the case of amoxicillin-clavulanate. More samples to be tested and some modifications or adjustments would be needed to validate the proposed methods.

Clinical applicability and feasibility into routine workflow are important considerations when deciding which method to implement in each laboratory. These methods would provide reliable results in daily routine work and the typical workflow would be that

performed in this study: first assess the Gram stain, and then perform the appropriate RAST only if true BSI is suspected.

The need for shorter diagnostic turn-around times has led EUCAST to develop a validated and standardized disc diffusion-based RAST directly from BC vials that can be implemented by any laboratory inexpensively. In this method proposed mid-2019, the species were assigned preliminary breakpoints for each of the reading times (4, 6 and 8 h) and a buffer area (ATU) between the susceptible and resistant categories was introduced to reduce the occurrence of false resistant and false susceptible results related to technical variation accelerated by the short incubation time. While the method is currently only validated for *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, *E. faecium*, and *S. pneumoniae* and for a limited panel of antimicrobials, work is underway to extend the method to more species (59).

The result of the Gram stain and early identification of the isolate through MALDI-TOF can be useful in addressing antimicrobial therapy based on the clinicians' epidemiological knowledge; nevertheless, among patients with BSI, the empirical treatment choice is complicated by the growing threat of antimicrobial resistance.

MALDI-TOF MS has completely revolutionized clinical bacteriology by dramatically accelerating the identification of microorganisms. Thus, after 3-6 hours of the initial subculture of positive BCs, it is possible to achieve in most cases (excluding yeasts and other slow-growing microorganisms) a reliable identification of the bacteria involved.

For determining the bacterial isolates' resistance profile, phenotypic antibiotic susceptibility testing is the gold standard method. Currently available phenotypic tests usually require up to 24 h to provide results. Rapid communication of the AST results has historically represented the challenge of clinical microbiologists. Having tools that allow to report antibiograms quickly and safely allows us to get out of obsolete empiricism.

Same-day results can be obtained using molecular techniques or ICT assays that allow the detection of resistance markers. However, although extremely useful (detection of ESBL, carbapenemase producing or MRSA strains) these techniques only provide information about the presence of certain genetic resistance markers and not phenotypic data. The goal of a RAST performed directly from positive BCs is to have same-day preliminary antibiotic susceptibility results, which is crucial in improving empirical antibiotic therapy. In fact, integrating results obtained with rapid identification technologies and susceptibility testing associated with ASPs has shown to improve outcomes in patients with BSI (135).

RASTs like the ones proposed here and that introduced by EUCAST are easy to perform and seem to provide reliable results (at least for certain antibiotics) within 4-6 h for the detection of *Enterobacterales*. If the Microbiology Department had afternoon operating hours, performing the RAST at about 11 a.m., it would be feasible to report provisional antibiograms at 4:00 p.m.

A recent survey of 209 laboratories in 25 European countries carried out by the ESCMID Study Group for Bloodstream Infections, Endocarditis and Sepsis (ESGBIES) revealed that only 4.7% (9/190) of laboratories validated and transmitted the results of identification

and AST of BC pathogens to clinicians 24 h/day. Results from this same study showed that the fundamental problem in the current diagnosis of BCs is insufficient service coverage by laboratories, despite the fact that BC diagnosis has been considered urgent, implying availability of laboratory services 24/7 (63). In the diagnosis of BSI and sepsis, support services, such as microbiology, tend to have a limited weekend on-call service. The study by Morton et al. suggests that this practice could lead to a less accurate diagnosis of sepsis (136).

Our Microbiology Department has limited operating hours, which prevents the report of results in the afternoon. In addition, by having satellite incubators from the moment a bottle becomes positive until it is received in the laboratory, a period of time passes, which further limits the reading of antibiograms in the same work shift. As mentioned in previous sections of this document, without human resources (that is, an on-call microbiologist to report results) methodological advances discussed in this section are completely ineffective.

Study limitations

The study has some limitations:

1. The number of samples evaluated was small.
2. Only *Enterobacterales* were evaluated. More studies are needed to evaluate these RASTs in other microorganisms frequently involved in BSI like NFGNB, *Staphylococcus* spp., *Enterococcus* spp. or *S. pneumoniae*.

3. The strains evaluated showed little diversity in the resistance mechanisms involved.
4. Further validation studies are required to improve the AST strategy directly from BCs.

OBJECTIVE 3

Analysis of the time-to-positivity of blood cultures during a two-year period: evaluation of real and contaminated episodes

This prospective study describes and evaluates the TTP of the first positive bottle of all episodes, real and contaminations, isolated during a two-year period. In total, 2890 episodes were evaluated.

In our study, 96.2% of the bottles belonging to true episode bottles became positive in ≤ 48 hours, so the results are in line with those previously published and support de-escalation of antibiotics after 48 hours if fungaemia or anaerobic BSI is not suspected (137). This information can be valuable in microbiology departments with limited operating hours like ours. A frequently asked question by physicians is the status of BCs of a specific patient if they have not yet become positive, and how long they have been incubating. Managing TTPs of each setting helps address this question by reliably indicating the probability that vials become positive, or even the type of microorganisms expected at a given TTP.

According to our results, no significant differences were found between the TTP of adults and children, although the number of paediatric BSI evaluated in the study was small. Historically, it has been believed that bacteraemia in the paediatric population was associated with a high bacterial load. However, and as stated before, the incidence of

low-level bacteraemia in these patients is presumed to be more common than has been reported and is estimated to occur in 38% to 68% of all paediatric patients with a positive BC (32,38,39).

The TTP of *Enterobacterales* and *Streptococcus* spp. was shortest and the TTP of *Candida* spp. was longest. Within *Enterobacterales*, *E. coli* produced significantly lower TTPs, and within streptococci, *S. pneumoniae* yielded significantly lower TTPs. These results are in line with other published data (138).

According to our results, if the Gram stain revealed Gram-positive cocci in clusters of flasks that had signalled positive in $\leq$ 12 h, the probability of it being *S. aureus* and not a CoNS was 73.7% (95% CI, 62.1-82.8%), which may suggest immediate additional therapeutic measures in such circumstances, e.g., withdrawal of a venous catheter and rules out contamination. A TTP greater than 24 h with visualization on the Gram stain of Gram-positive cocci in chains practically rules out *S. pneumoniae*. For Gram-negative bacilli, a TTP of ≤ 12 h suggested that the involvement of a non-fermenting organism was very unlikely. Comparable results have been reported in previous studies (139).

Based on what is stated in the previous section and as Ning et al. stated in their article (138), TTP can undoubtedly influence the use of antibiotics. We can adjust the antibiotics based on the characteristics of the Gram stain and TTP after a flask signals positive. Thus, when Gram-positive cocci in clusters are visualized in the Gram stain belonging to vials with high TTPs, it will be highly likely to be contamination, while for the same staining with short TTPs (less than 12 hours) it would be necessary to act. Therefore, TTP has value for rational antibiotics usage.

As Martínez et al. exposed in their work (139), it makes no sense to make predictions about the origin of bacteraemia based on TTP without considering the microorganism involved. In our study, no significant differences were found in TTP belonging to patients with urinary tract infection and patients with GI and biliary infection and the same result was obtained when only *Enterobacterales* were evaluated. These results reinforce the idea that for most cases the TTP depends more on the type of microorganism involved than on the source of infection.

BCs remain a cornerstone in the diagnosis of infectious endocarditis and several studies have focused on the potential tool of TTP in its diagnosis and prognosis (140,141). In this study, and similar to others (140,142,143), the TTP in *S. aureus* episodes was significantly lower in infectious endocarditis than in the rest of the episodes with a different source. However, we did not find this result in the case of episodes produced by *E. faecalis* and CoNS. Oldberg et al. found that in *E. faecalis* bacteraemia low TTPs were associated with infective endocarditis and suggested that low TTPs could be used to help determine the need for echocardiography in patients with this condition (144). In our study, we did not find such a relationship for this species, although the sample size was much smaller than that of the aforementioned study.

Multiple studies have reported on the association between TTP and clinical outcome across multiple microorganisms with conflicting results. In the multicentre randomized controlled trial conducted by Hamilton et al. which aimed to quantify the relationship between the TTP of monomicrobial BCs and mortality, the authors did not find robust evidence of a relationship between mortality and TTP in staphylococci (both coagulase-

negative and *S. aureus*), *Pseudomonas* spp., enterococci, *Bacteroides* spp., and all members of the *Enterobacterales*. For *Candida* spp., they curiously identified a relationship between increasing TTP and mortality (99). Based on this study, we focused on the evaluation of the 28-day mortality in the candidaemia episodes, finding no relationship between TTP mortality. However, the number of episodes evaluated in our study was small and more data would be required to confirm these results.

Few articles evaluate the TTP of contaminations or real episodes produced by CoNS. The TTP of real episodes was significantly lower than that of the episodes considered contaminations. Our institution must tackle with a high contamination rate despite great efforts being made to avoid it. As mentioned in other sections of this document, BCs should be interpreted from a global patient perspective, so that to assess whether a BC is contaminated, different aspects should be considered: clinical findings and patient's condition determined by infectious diseases physicians on the one hand, and type of microorganism involved and number of positive BC bottles and sets, among others, judged by microbiologists on the other. But, even following both clinical and microbiological criteria, the interpretation of positive BCs for CoNS is often troublesome (111). Therefore, having another tool that can shed some light on the role the isolated microorganism plays in the episode is especially important. In this way, the logistic regression model fitted in Excel can be a useful instrument that allows the user to play with the variables (TTP, PBDP) to see how each variable impacts in BCC and determine the probability of contamination in each case. The proposed model could be improved by incorporating multiple independent variables such as age of the patient, requesting department or BC extraction through lines depending on each institution which would

allow its implementation in the daily routine practice helping to elucidate problematic cases.

Different definitions of TTP can be found in the literature. While some authors define the TTP as the time between collection of BCs and the positive signal, others consider it the time from the start of incubation to a positive signal. As our institution has several satellite incubators operating 24/7, time between extraction and incubation is noticeably short and therefore the latter definition was used. In any case, given the variability that that is known to exist across centres (99), their protocols, and their BC incubation systems, in clinical daily practice, each institution and, by extension, each the ASP should be familiar with their own TTP, regardless of the definition followed.

TTP may provide early clues about the microorganism involved and its involvement in the episode. In hospitals with a high contamination rate, BLR formula based on data from each hospital could provide an objective and easy tool with effective diagnostic performance, which could help determine the role CoNS plays in infection.

Study limitations

The study has some limitations:

1. The sample size was small and the number of paediatric BSI evaluated was scarce.
2. With the exception of the episodes of candidaemia and those with TTP> 24 h, the severity of infection and mortality were not evaluated. Underlying diseases such as haematological or oncological processes were not evaluated, either. Only immediate admission to the ICU after an initial evaluation in the ED and BC

extraction was examined as a criterion of severity. Flasks belonging to these patients (113) flagged positive significantly faster (P<0.0001) than in the rest of the patients with real BSI. As mentioned before, several previous studies have reported conflicting results on the association of TTP and clinical outcome in different bacteria and fungi (143–147). In a large prospectively collected multicentre study, TTP was not associated with mortality, except in *Candida* spp., where, unexpectedly, longer times were associated with worse outcomes (99).

3. Previous use of antimicrobials has shown to have great impact on TTP (148) but in our study was only evaluated for episodes with TTP > 24 h. Nevertheless, 66.2% of the BCs were drawn in the ED, where the extraction is routinely carried out before the patient receives any antibiotic treatment.

OBJECTIVE 4

Impact of the SARS-CoV-2 pandemic on blood culture utilization and characteristics of bacteraemia in SARS-CoV-2 patients

This short study aimed to evaluate the impact of the SARS-CoV-2 pandemic on BC utilization and describe the characteristics of bacteraemia in SARS-CoV-2 patients.

The surge of febrile patients to our network of hospitals led to a sharp and dramatic increase in the number of BCs requested, with a marked peak in March. This phenomenon has been described in other works. In fact, the one published by Sepulveda et al. even suggested a reduction of the incubation time from five to four days to avoid overwhelming the capacity of automated BC instruments (47). Despite the increase in requested BCs experienced, in our laboratory it was not necessary to decrease the incubation time. Conversely, in the study carried out by Mormeneo Bayo et al. in which the impact of the pandemic on bacteraemia and the use of BCs in another Spanish tertiary hospital was evaluated, a reduction in the rate of BCs processed was obtained, which they justify by the decrease in the number of patients who attended the ED, as well as by the decrease in scheduled surgeries caused by the pandemic (149).

The high contamination rate observed during the outbreak of the pandemic has widely been reported in other articles (47,149–152) and is likely attributable to:

- the increase in the number of BCs requested

- over-ordering in a population with a low incidence of true BSI
- the heavy workload
- the initial shortages of personal protective equipment experienced in early stages of the pandemic
- unfamiliarity of additional personal protective equipment worn by healthcare nurses taking BCs
- the high workload for the care of SARS-CoV-2 patients (oxygenation adjustment, prone positioning of the patients, among others) could have complicated the extraction of BCs.

In any case, contamination rates observed and reported in both 2019 and 2020 was high enough for the ASP to promote in 2021 the formation of a multidisciplinary team made up of microbiologists, infectologists, intensivists, preventivists, and nurses to address this issue.

The dramatic increase in the linezolid resistance rate experienced in 2020 compared to 2019 (from 8.9 to 25% [*P*=0.003]) makes it necessary to implement strict antimicrobial administration guidelines (especially in the ICU) to protect against further evolution of resistant mutants. As mentioned before, in order to comprehensively understand the current resistance mechanisms among linezolid-resistant clinical isolates belonging to BSI episodes, strains isolated during the last two years of the study (2019-2020) have been collected and are going to be further analysed.

Among patients with positive BCs, SARS-CoV-2 positive patients had a significantly higher proportion of HO BSI (82.7%) than non- SARS-CoV-2 patients (22.7%) (*P*< 0.001). In SARS-

CoV-2 positive patients, the catheter-related bacteraemia was the main source of bacteraemia, and the ICU the predominant requesting department. For SARS-CoV-2 negative patients, in contrast, the main source was the urinary tract, and the main requesting department was the ED. These results are in line with those published by Mormeneo Bayo et al. (149).

We found a low frequency of BSI in SARS-CoV-2 hospital presentation. In only two patients the source of BSI was the respiratory tract (one bronchoaspiration in the case of a patient with Parkinson's disease, and one pneumonia in the case of a patient with chronic obstructive pulmonary disease). In the rest of the SARS-CoV-2 patients with bacteraemia on admission, the source was the urinary, the GI tract or remained unknown. Our results are in agreement with those published by Engsbro et al. (153), which end up supporting a restrictive use of antimicrobials in patients with COVID-19 on admission.

In brief, our results show that in patients with SARS-CoV-2, bacteraemia on admission was rare and that the profile of isolated microorganisms in BCs in inpatients was that expected for patients with long hospital stays, suggesting that procedures associated with care were more likely causes of BSI than infection with SARS-CoV-2 per se.

The pandemic has highlighted the urgent need for close collaboration between ASP and infection prevention programs to optimize available resources, control the increase in contamination rates, and monitor nosocomial infections and antimicrobial overuse.

CONCLUSIONS

Among the conclusions of this doctoral thesis, we would like to highlight eight of them, which are the following:

1. In the present study we emphasize the continuous and progressive increase in BSI episodes at the HUA (tertiary hospital).
2. There has been a rise in the occurrence of candidaemia cases in HAD (district hospital) over the last years. A shift towards non-albicans *Candida* species is observed.
3. Our results reveal low level resistance rates to common antibiotics and antifungals in our area. Nevertheless, increasing resistance to linezolid in Gram positive cocci and to amoxicillin-clavulanate in BFG is becoming a real threat.
4. The AST methods proposed and interpreted with standard breakpoints have shown to be useful for cephalosporins and carbapenems, so for *Enterobacterales* it seems feasible to generate a preliminary AST report in five hours. However, for amoxicillin-clavulanate the CA was low in both proposed methods.
5. Our results support de-escalation of antibiotics after 48 hours if fungaemia or anaerobic BSI is not suspected.
6. The TTP is a useful tool in guiding towards the type of microorganism involved and its role in infection.
7. The logistic regression formula based on TTP data from each hospital could help determine the role CoNS plays in infection and reduce unnecessary antimicrobial

treatments, avoidable additional tests, increased length of hospital stay and unnecessary removal of lines.

8. During the SARS-CoV-2 pandemic although the increase in BC ordered did not collapse the automated incubators at any time, the increase in contaminated BCs posed a challenge when interpreting BC results.

Entre las conclusiones de esta tesis doctoral, recalcamos ocho de ellas, que son las siguientes:

1. En el presente estudio destacamos el incremento continuo y progresivo de episodios de bacteriemia en el HUA (hospital terciario).
2. Ha habido un aumento de casos de candidemia en el HAD (hospital comarcal) en los últimos años. Se observa un cambio hacia especies de *Candida* no albicans.
3. Nuestros resultados revelan un bajo nivel de resistencia a los antibióticos y antifúngicos frecuentemente utilizados en nuestra área. Sin embargo, el aumento en la resistencia a linezolid en cocos Gram positivos y a amoxicilina-clavulánico en *B. fragilis* Group se está convirtiendo en una amenaza real.
4. Los métodos de antibiograma propuestos e interpretados con puntos de corte estándar han demostrado ser útiles para cefalosporinas y carbapenémicos de manera que para enterobacterias parece factible reportar un antibiograma preliminar en cinco horas. Sin embargo, para amoxicilina-clavulánico, los resultados obtenidos fueron subóptimos para ambos métodos.
5. Nuestros resultados apoyan la desescalada antimicrobiana transcurridas 48 horas siempre que no se sospeche de fungemia o bacteriemia anaerobia.
6. El tiempo de positivización constituye una herramienta útil para orientar hacia el tipo de microorganismo aislado y su papel en la infección.
7. La fórmula de regresión logística basada en los datos de tiempo de positivización de cada hospital podría ayudar a determinar el papel que juegan los estafilococos coagulasa-negativos en la infección y, así, evitar tratamientos antimicrobianos

innecesarios, pruebas diagnósticas adicionales, incrementos en la estancia hospitalaria y cambios innecesarios de vías.

8. En la pandemia SARS-CoV-2 el aumento de los hemocultivos solicitados no colapsó los incubadores automáticos en ningún momento, pero el aumento de hemocultivos contaminados planteó un desafío a la hora de interpretar los resultados.

APPENDICES

Appendix I

The two brief cases described below have been accepted for publication by the Enfermedades Infecciosas y Microbiología Clínica. Ref. EIMC-D-21-00508R2.

Gonococcal bacteraemia: report of two clinical cases linked with pharyngeal asymptomatic infection

Disseminated gonococcal infection (DGI) remains rare, is often preceded by asymptomatic mucosal infection and can be present in the absence of urogenital infection (154,155).

Case 1

A 31-year-old previously healthy woman was admitted to the ED with persistent odynophagia and fever. On admission her temperature was 37.7 °C. Physical examination revealed millimetre papules in both lower extremities. The oropharynx was normal, without tonsillar exudates or adenopathies. Blood analysis showed white blood cells of 11900/mm3 with 83.5% neutrophils. No signs and symptoms related to the genitourinary system were found. BC and a pharyngeal sample were collected, and the patient was discharged with amoxicillin/clavulanate (875/125mg).

Case 2

A 51-year-old man presented to the ED with a three-day history of fever, polyarthritis, and non-specific skin lesions on the right foot, left leg and right wrist. The day before the

onset of symptoms he had received the first dose of hepatitis B vaccine. An adverse reaction to the vaccine was suspected and the patient was admitted to our hospital. Physical examination revealed temperature 38.1 °C and BC were drawn.

Aerobic BC vials flagged positive and Gram-negative diplococci were observed on Gram stain. After 18h of incubation in 5% CO2 at 35 °C, greyish colonies grew on chocolate agar that were identified as *N. gonorrhoeae* using MALDI-TOF MS (Bruker Daltonics).

WGS was performed using the MiSeq platform. Assembled genomes of the isolates were input for the ResFinder 3.2 tool in the CGE website (https://cge.cbs.dtu.dk) for identification of acquired antimicrobial resistance genes and chromosomal mutations. The sequence data have been submitted to European Nucleotide Archive (PRJEB37804). Table 37 shows the genotypes, MICs, and antimicrobial resistance determinants of the two isolates.

Given the results, both patients were started on cefixime 400 mg/12 h. Serologic tests for HIV, hepatitis A, B, and C viruses, and *Treponema pallidum*, as well as culture and PCR (AnyplexTM STI-7, Seegene) on endocervical (patient 1) and urethral (patient 2) specimens were performed yielding negative results.

On direct questioning, patient 1 revealed having had her last sexual intercourse two months ago, having become pregnant and having undergone an elective abortion four weeks after. She also admitted having had oral sex.

Patient 2 acknowledged risk sexual behaviour including unprotected oral sex with men and women. DNA of *N. gonorrhoeae* was detected in the pharyngeal smears of both patients. Both patients fully recovered after 4 days of treatment.

DGI is generally characterized by a triad of symptoms known as “arthritis-dermatitis” syndrome (cutaneous lesions, tenosynovitis, and arthralgia) and genitourinary symptoms are usually lacking. However, patients like the one in the first case, may present with nonspecific symptoms like fever, malaise, or myalgia.

DGI remains rare and is stated to be more frequent in women since in them primary infection is frequently asymptomatic and, therefore, untreated (156,157). Haematogenous spread is thought to occur 2-3 weeks after primary infection and affects 0.5-3% of infected individuals. Host-dependent factors as well as inherent features of the strain involved might be involved. Menstruation, pregnancy, HIV infection, systemic lupus erythematosus, host complement deficiency, splenectomy or the use of intrauterine devices have been postulated as possible triggers of the infection spread beyond local infectious sites (158–160). A failure of CEACAM3-mediated innate detection might be linked to the ability of gonococci to cause disseminated infections (161). Nevertheless, no single genetic locus has been identified yet as definitive cause of DGI (162).

In our study, both patients suffered an alteration of the immune system (pregnancy and vaccination) that could have triggered the spread to the bloodstream. The pharynx has shown to act as an important reservoir for *N. gonorrhoeae* (163), and, although it usually remains asymptomatic, the patient in case 1 presented odynophagia that could not be attributed to any other causative agent.

Clinicians should be aware of the signs and symptoms of DGI, including cutaneous manifestations of this condition and of the fact that absence of genitourinary symptoms

does not rule out the diagnosis. Prompt recognition and treatment of pharyngeal asymptomatic carriers will have a positive effect to control the burden of gonorrhoea.

Table 37. Genotypes, MICs, and antimicrobial resistance determinants of the two isolates.

ISOLATE	GENOTYPE		SUSCEPTIBILITY (mg/L)						CHROMOSOMAL MUTATIONS									
	MLST	NG-MAST	BEN	CIX	CTA	CIP	TET	AZI	PBP2	PBP1	PorB	GyrA	ParC	S10	mtrR promoter	MtrR	MtrD	23S rRNA
1	9363	6765	0.5	0.016	0.064	0.016	4	2	Type II non-mosaic	-	G120K, A121N	-	-		-	-	S821A, K823E	-
2	7822	14994	0.38	0.016	0.023	2	1	0.25	Type V non-mosaic	L421P	-	S91F, D95A	S87R	V57M	-	A39T	-	-

MLST: multilocus sequence typing, BEN: benzylpenicillin, CIX: cefixime, CTA: cefotaxime, TET: tetracycline, AZI: azithromycin.

Appendix II

Adapted from: Aguirre Quiñonero A, Calvo Muro FE, Lodoso Torrecilla B, Canut Blasco A. Early-onset neonatal pneumococcal sepsis and meningitis. Published January 2019 by the Journal of Clinical Neonatology.

Case report: early-Onset Neonatal Pneumococcal Infection

A 1-day-old male infant presented with continuous crying, expiratory moan, and low-grade fever. He was vaginally born at term to a 37-year-old mother, weighed 2670 g and the Apgar scores were 9/10. Perinatal infection was suspected prompting admission to the Neonatal Unit. At the time of admission, he presented with seizures, stiffness, and cyanosis. Lumbar puncture as well as BC were drawn, and the patient was empirically started with cefotaxime and ampicillin.

No family epidemic environment was described nor any history of fever, chorioamnionitis or prolonged rupture of membranes had been reported. In fact, the pregnancy had been uncomplicated. Mother rectal and vaginal *S. agalactiae* carriage performed in the third trimester were negative and the HIV test was likewise negative. Before and after delivery she did not show any signs or symptoms of infection nor other clinically important symptoms.

The initial laboratory findings included leukopenia (3700 cells/mm3) and increased C-reactive protein (150 mg/L). Biochemical analysis of the cerebrospinal fluid could not be

carried out since only samples for the microbiology laboratory were taken. In the Gram stain, Gram-positive diplococci were observed and after 24 hours of incubation, *S. pneumoniae* was identified using MALDI-TOF MS. After 24 hours of incubation, BCs signalled positive, and the same bacterium was recovered.

MICs were determined by the microdilution method and following 2019 EUCAST interpretative criteria, the isolate was susceptible to penicillin (MIC 0.03 mg/L), cefotaxime (MIC < 0.06 mg/L), vancomycin (< 0.5 mg/L), and erythromycin (MIC < 0.25 mg/L).

The serotype determination was carried out and the serotype 23F was identified.

The patient continued with seizures for the next ten hours, which required two phenobarbital loading doses and subsequent maintenance treatment. The patient remained hemodynamically stable without repercussion to other organs and systems. Imaging tests were performed without detecting any complication.

Once the Gram stain result was known, ampicillin was switched to vancomycin until the result of the antibiogram was available; then, descalation with cefotaxime was carried out until the end of treatment.

A vaginal culture of the mother was performed which was negative for *S. pneumoniae*.

The infant improved and was discharged after four weeks of hospitalization. After discharge, a bilateral deafness and a certain psychomotor retardation were noted. At

present, the patient is followed-up by neuropaediatrics, the Early Care Unit, and the Otorhinolaryngology Department.

S. pneumoniae invasive neonatal infection is rare in developed countries but is associated with high morbidity and mortality (35-54%) rates (164,165). Infections in the newborn usually occur during the second or third week of life; early onset infection occurring during the first 72 hours of life is less frequent and has a worse prognosis than late onset one (166).

Clinical features of pneumococcal neonatal sepsis are indistinguishable to those produced by *S. agalactiae* sepsis, and thus is usually not suspected in the newborn (167,168).

Four possible mechanisms for which *S. pneumoniae* may reach the foetus or neonate have been postulated: transplacental route; ascending infection from genital tract; passage through colonized birth canal; or postpartum respiratory spread (165,169). In the case exposed, the exact source of infection remained unknown. The vaginal culture performed on the mother after delivery was negative for *S. pneumoniae* and she did not have any signs or symptoms of infection before or after delivery. On the other hand, nosocomial transmission was unlikely since personal protective equipment was used among the health personnel following mandatory compliance rules.

Gestational age, prematurity, prolonged rupture of membranes, low birth weight nor gender have been associated with early-onset *S. pneumoniae* infection. So far, known risk factors include vaginal delivery and high pneumococcal colonization (165). In order to

prevent mother-to-child transmission, associated risk factors should further be studied to shed some light on this disease.

Clinical features of neonatal *S. pneumoniae* infection are similar to those of early-onset *S. agalactiae* infection making it almost impossible to differentiate these two diseases on a clinical basis. Whereas *S. agalactiae* is commonly isolated from the female genital tract, *S. pneumoniae* is not part of the normal vaginal flora and is infrequently isolated from vaginal samples. The rate of urogenital colonization with pneumococci is also low (169). Colonization of the genital tract can occur after orogenital contact by a carrier or in the context of *S. pneumoniae* upper respiratory infection. Compared to *S. agalactiae*, maternal pneumococcal carriage is not routinely investigated. However, in view of the case exposed and similar to other authors, published data state that vaginal carriage of *S. pneumoniae* among pregnant women could be considered (169–171).

In our case, the isolate was susceptible to both penicillin and cefotaxime and the patient fully recovered after cefotaxime treatment. However, in areas where antimicrobial-resistant *S. pneumoniae* is prevalent, the addition of vancomycin and/or larger dosage of cefotaxime can be considered if *S. pneumoniae* infection is suspected.

The serotype of the isolate was 23F and is included in PVC13. Although there is insufficient evidence to recommend pneumococcal vaccination during pregnancy (172) maternal immunization with PVC13 in the third trimester could provide passive protection to the neonate and thus, reduce neonatal infections.

PUBLICATIONS AND PRESENTATIONS

List of publications and presentations while doing the doctoral thesis (only as fist author)

- Aguirre Quiñonero A, Alonso R, Marroyo-Salazar M, Canut-Blasco A. Gonococcal bacteraemia: report of two clinical cases linked with pharyngeal asymptomatic infection. Accepted for publication by the Enfermedades Infecciosas y Microbiología Clínica. Ref. EIMC-D-21-00508R2.
- Aguirre Quiñonero A, Calvo Muro FE, Lodoso Torrecilla B, Canut Blasco A. Early-onset neonatal pneumococcal sepsis and meningitis. Published January 2019 by the Journal of Clinical Neonatology.

31st ECCMID (European Congress of Clinical Microbiology and Infectious Diseases) Congress:

- Aguirre Quiñonero A, Lecaroz Agara MC, Hernáez Crespo S, Gómez González C, Almela-Ferrer MR, López Mirones JI, Cordón Rodríguez MLA, Rodríguez Achaerandio A, Canut Blasco A. Anaerobic bacteraemia: aetiology and antibiotic susceptibility profile during a 6-year period.
- Aguirre Quiñonero A, Lecaroz Agara MC, Hernáez Crespo S, Gómez González C, Almela-Ferrer MR, López Mirones JI, Cordón Rodríguez MLA, Canut Blasco A. Impact of the SARS-CoV-2 pandemic on blood culture utilisation and bacteraemia in a tertiary hospital in Northern Spain.

30th ECCMID Congress:

- Aguirre Quiñonero A, Marroyo-Salazar M, Saez De Adana Arroniz E, Canut A. Time to positivity of blood cultures and its role in the diagnosis of bacteraemia.
- Aguirre Quiñonero A, Rodríguez A, Solinís MA, Canut A. Pharmacokinetic/pharmacodynamic analysis of tedizolid phosphate compared to linezolid for the treatment of infections caused by Gram-positive bacteria.

XXIV Congreso de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC):

- Aguirre Quiñonero A, Calvo Muro FE, Lecaroz Agara MC, Hernáez Crespo S, Gómez González C, Rodríguez Achaerandio A, López Mirones JI, Cordón Rodríguez MLA, Canut Blasco A. Bacteriemia por *Escherichia coli* y el grupo ESKAPE: incidencia y tendencias en el Hospital Universitario de Álava.
- Aguirre Quiñonero A, Calvo Muro FE, Lecaroz Agara MC, Hernáez Crespo S, Gómez González C, Rodríguez Achaerandio A, López Mirones JI, Cordón Rodríguez MLA, Canut Blasco A. Candidemia: epidemiología, distribución de especies, tiempo de positividad y sensibilidad en el Hospital Universitario de Álava.
- Aguirre Quiñonero A, Calvo Muro FE, Lecaroz Agara MC, Hernáez Crespo S, Gómez González C, Rodríguez Achaerandio A, López Mirones JI, Cordón Rodríguez MLA, Canut Blasco A. Evaluación del rendimiento de la prueba del antígeno neumocócico en orina en pacientes con bacteriemia por *Streptococcus pneumoniae*.

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