Biofilm-forming microorganisms causing hospital-acquired infections from intravenous catheter: A systematic review

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ABSTRACT

The high prevalence of nosocomial infections is related to the use of medical insertion devices such as central venous catheters (CVCs). Most of the microorganisms causing nosocomial infections are biofilm producers, this characteristic allows them to adhere to abiotic surfaces and cause initial catheter infections that can lead to bloodstream infections. Our main goal in this systematic review was to evaluate the prevalence of biofilm among CVC-related infections, particularly among Intensive Care Unit (ICU) patients, in the studies applying different *in vitro* and *in vivo* methodologies.

All studies reporting clinical isolates from patients with catheter-related nosocomial infections and biofilm evaluation published up to 24 June 2022 in the PubMed and Scopus databases were included. Twenty-five studies met the eligibility criteria and were included in this systematic review for analysis. Different methodologies were applied in the assessment of biofilm-forming microorganisms including *in vitro* assays, catheter-infected *in vitro*, and *in vivo* mouse models. The present study showed that between 59 and 100% of clinical isolates were able to form biofilms, and the prevalence rate of biofilm formation varied significantly between studies from different countries and regions. Among the clinical isolates collected in our study set, a wide variety of microorganisms including Gram-positive strains, Gram-negative strains, and *Candida albicans* were found. Many authors studied resistance mechanisms and genes related to biofilm development and surface adherence properties. In some cases, the studies also evaluated biofilm inhibition assays using various kinds of catheter coatings.

Introduction

The prevalence of hospital-acquired or nosocomial infections exceeds 25% in developing countries and up to 15% in developed countries, resulting in the death of approximately 40,000 hospitalized patients worldwide (Lemiech et al., 2021). The insertion of medical devices in the hospital setting for various patient treatment purposes has increased the incidence of nosocomial infections, including the various types of central venous catheters (CVCs) used for patient therapy and even for outpatients receiving various types of treatment such as hemodialysis, minor surgeries, some cancer treatments, among others [1](Baier et al., 2020).

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[1] Some references seem to have sometimes a space after the first "(". Please check this typo. Thank you.

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CVCs are used to provide nutritional support to hospitalized patients, administer fluids and medications, and monitor hemodynamics, so their contamination with various microorganisms can lead to bloodstream infections that are a major cause of mortality, increasing the length of stay of hospitalized patients and Intensive Care Unit (ICU) stays and leading to additional health care costs (Brunelli et al., 2018). Nosocomial infections caused by catheterization procedures are frequently associated with antimicrobial-resistant microorganisms including various enterobacterial species, *Enterococcus* spp., *Staphylococcus* spp., and fungi such as *Candida* spp. (Liao et al., 2021).

Between 50 and 70% of nosocomial infections are caused by biofilm formation on implanted medical devices such as CVCs (Asker et al., 2021). Biofilms are made up of colonies of a wide variety of microorganisms bound by an extracellular matrix and can adhere to different surfaces such as CVCs (Muhsin Jamal et al., 2018). It is known that almost all bacteria and some fungal species have the inherent ability to form biofilms that allow them to evade the host immune response and tolerate treatment with a wide range of antibiotics and antifungals becoming a serious public health threat (Roy et al., 2018).

Therefore, this systematic review aimed to analyze studies comprising a variety of *in vitro* and *in vivo* methodologies used to study the biofilm formation of different microorganisms from nosocomial infections related to the use of central intravenous catheters.

Materials and methods

For a better understanding of the involvement of biofilm within nosocomial infections caused using CVCs, the relevant literature was reviewed. This study was conducted following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines by three independent reviewers (SPC-P, ALÑ-T, and AM). In each electronic database, a combination of MESH terms was used to conduct the search applying the following strategy: "((biofilms) AND ((microbial resistance) OR (drug resistance)) AND ((cross infection) OR (nosocomial infection)) AND ((intravenous) OR (catheters)))". To start with the article collection each reviewer included articles related to the main topic, involving catheters and biofilms as keywords. All studies published on Scopus and PubMed databases until 24th June 2022 were retrieved. During the identification step in the databases, 248 results were found. When screening the studies set, 20 duplicate studies were removed.

The data selection for eligibility criteria from the remaining 228 articles was limited to human clinical isolates and studies in English, excluding articles in other languages (n=16), reviews (n=63), nonhuman studies (n=66), and records with irrelevant or unquantified information, editorials, congress, and meeting abstracts were also removed (n=22). In total, 167 articles were excluded from the study set. At each level, the reviewers independently screened the articles and finally merged their conclusions. An additional examination of the selected articles was realized by the remaining reviewers (AM, JR, and DG-C) focused on the homogeneity of the eligibility criteria of previous reviewers in the initial data set. Discrepancies were resolved by discussion before finalizing the records for the evaluation of eligibility criteria. In case of disagreements, the third assessor (AM) was assigned to make a final decision.

In the eligibility step, the main inclusion criteria were the evaluation of biofilm formation and the prevalence of biofilm-related infections, including observational studies (more exactly, cohort, retrospective, and case-control studies). Furthermore, data regarding the geographical location of the study set, and the use of antimicrobial agents in clinical isolates were also extracted from the studies. All studies without information about biofilm formation or clinical isolates were consequently excluded. The method to quantify biofilm biomass was not a criterion to include or exclude any paper in this systematic review. Therefore, 25 of 61 articles were chosen. Finally, the information from the final 25 articles was analyzed and extracted in the present study.

The extracted information included the first authors' names, time of the study, year of publication, location, sample size, microorganism identification (species), biofilm formation rate, type of study, and the type of biofilm. The initial three authors (SPC-P, ALÑ-T, and LJE-M) extracted all data, and further confirmation and final evaluation were realized by the lead authors (AM, JR, and DG-C).

Results

Study inclusion criteria and characteristics of the eligible studies

A total of 248 articles were retrieved from PubMed and Scopus based on the established search terms. Following the article selection process, 25 articles that met the inclusion criteria were included for full-text analysis. The PRISMA flow chart summarizing the search strategy is illustrated in Fig. 1. This systematic review does not include time or location delimitations, so the data collected includes studies covering different regions of the world at different times. All articles were carefully reviewed for the extraction of relevant information related to biofilm formation on intravenous catheters in patients with hospital-acquired or nosocomial infections.

General effects of biofilms in nosocomial infections

The overall data from the selected studies are shown in Table 1 comprising studies conducted between 2001 and 2020 in several countries around the world from Europe (7/25), Asia (6/25), South America (6/25), North America (5/25), and Africa (1/25). In addition, several methodologies such as *in vitro* assays (14/25), *in vitro* catheter-infected assays (10/25), and *in vivo* mouse catheter infection models (2/25) were used to assess biofilm formation. The tested strains of different bacterial and fungal species were isolated from intravenous catheters, medical devices, and clinical sites, where 59 and 100% of the clinical isolates were able to form biofilms.

Characteristics of catheterized patients with nosocomial infections

From the 25 articles analyzed in this review, all samples came from patients with nosocomial infections, but some of them reported extra information about the patients. The data collected on catheterized patients are summarized in Table 2. The most common aspect of the population set is the provenience of clinical isolates from the ICU. Moreover, studies in Bolivia and the Czech Republic showed the highest rates of nosocomial infection (Cerezales et al., 2018; Olejnickova et al., 2014). However, only studies in Bolivia and Poland evaluated the correlation between the nosocomial infection rate and the use of antibiotics in all ICU patients (Cerezales et al., 2018; Sobczak et al., 2019).

Although all samples were collected from nosocomial infections, some strains were directly isolated from catheters (Mekni et al., 2012) and other isolates came from clinical samples of nosocomial infections and subsequently were cultivated and analyzed into infected catheters (Ramos et al., 2019). Concerning gender, the recollected data showed a higher rate of nosocomial infections in men. Finally, as expected, the age range of most patients with nosocomial infections was neonates/infants (below one year) children, adults, and geriatric patients (up to 100 years of age).

Geographical distribution of biofilm-forming

It is well-known that biofilms are the dominant form of growth in almost all bacteria species across the world being associated with the development of nosocomial infections through catheter insertions (Olejnickova et al., 2014). The prevalence rate of biofilm-related infections significantly varied among studies of different regions and countries. As shown in Table 3, the higher prevalence rate of biofilm was reported in Africa, followed by South and North America. On the other
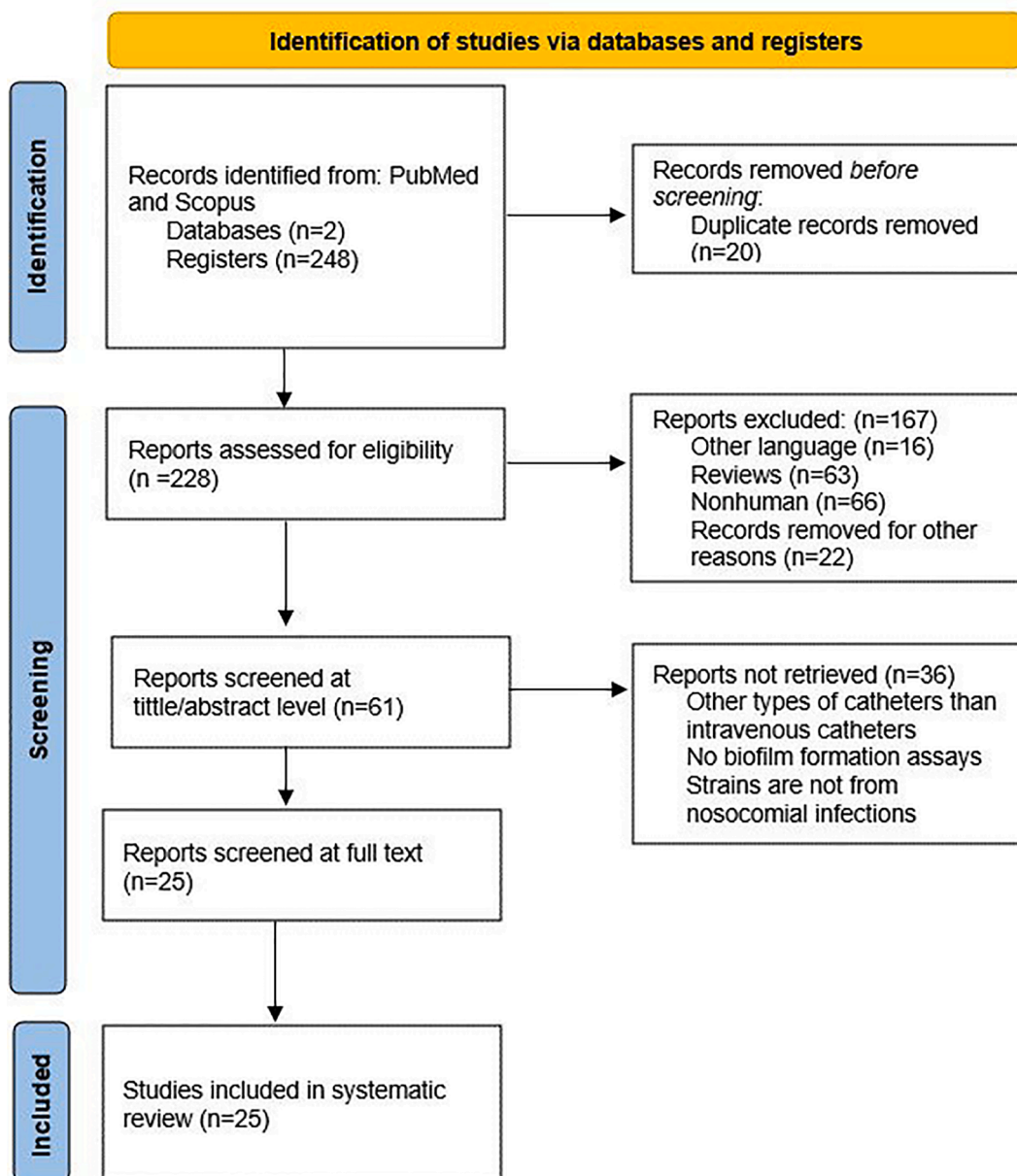
Fig. 1. Prisma flow chart of included and excluded studies of the selection process.

hand, Asia reported a lower prevalence of biofilm. Nonetheless, it is important to mention that the number of studies per region significantly varied, which could easily lead to erroneous conclusions, and further studies are essential to confirm these results.

In Fig. 2, the study set is shown by country and most countries only reported one study, except for Italy (2 studies), India (3 studies), USA (4 studies), and Brazil (5 studies).

It is also remarkable how the prevalence of biofilm rate decreased in comparison to other countries of the same region with similar features, suggesting that more studies are needed to evaluate the real biofilm prevalence worldwide among nosocomial infections.

In vitro studies

Due to their ability to form biofilms, crystal violet methodology is usually the gold standard procedure to measure biofilm biomass. This methodology consists of the adjustment of bacterial growth cultures to a specific optical density or colony-forming unit (CFU)/mL value

incubating the initial bacterial concentration into 96-well polystyrene plates containing a certain medium with some complements (Olejnick-ova et al., 2014). After 24h or 48h of incubating at 37°C, the media culture and bacterial planktonic cells are removed through multiple washes. Subsequently, the absorbance is measured at a certain wavelength. Although other methodologies could be applied as shown in Table 4, these techniques are usually a qualitative evaluation (such as Qualitative Detection, QD) or a quantitative evaluation less applied in biofilm research, such as Quantitative Biofilm Analysis (QBA), test for slime production, and safranin staining. The most trustful evaluation of the ability to form biofilm is its classification as weak, moderate, and strong biofilm former by the Biofilm Formation Index (BFI). However, the parameters for this classification varied between studies being a pitfall that must be solved to allow comparing data in the literature.

As shown in Table 4, all studies confirmed the ability of the clinical isolates to form biofilms and four of the studies tried to classify these biofilms. In addition, Sohail and Latif were able to classify 208 MRSA strains, from which 50% were weak, 27% were moderate, and 23% were

Table 1

General information extracted from the data set selected for the present systematic review.

Study	Region	Country	Study type	Biofilm rate, n (%)	Species	Type of isolated
(Souza et al., 2019)	South America	Brazil	CI - *In vitro*	4/4 (100)	*Corynebacterium striatum* MDR and MDS	CS
(Burton et al., 2006)	North America	Canada	CI - *In vitro*	6/6 (100)	*Escherichia coli* P18, *Pseudomonas aeruginosa* PA01, *Staphylococcus epidermidis* 1457, *Klebsiella pneumoniae* P30, *Proteus mirabilis* 6285, and *Enterococcus faecalis* 36171	C
(Sohail & Latif, 2018)	Asia	Pakistan	*In vitro*	203/344 (59)	MRSA	CVC
(Sobczak et al., 2019)	Europe	Poland	CI - *In vitro*	15/15 (100)	*Staphylococcus haemolyticus, S. capitis, S. epidermidis, S. cohnii,* and *Klebsiella pneumoniae*	UAC, UVC
(Hoque et al., 2016)	Asia	India	CI - *In vitro*, MMCI - *In vivo*	2/2 (100)	*Staphylococcus aureus* y *E. coli*	N/A
(Jain et al., 2016)	North America	USA	*In vitro*	18/24 (75)	*Acinetobacter baumannii*	CVC
(Sharma et al., 2011)	Asia	India	*In vitro*	61/100 (61)	Coagulase-negative staphylococci (CoNS)	CVC, CS
(Ramos et al., 2019)	South America	Brazil	CI - *In vitro*	2/2 (100)	*C. striatum* MDR	CVC, CS
(Sued et al., 2017)	South America	Brazil	CI - *In vitro*	5/6 (83)	Oxacillin-resistant *S. haemolyticus*	MD
(Cerezales et al., 2018)	South America	Bolivia	*In vitro*	3/3 (100)	*A. baumannii*	CVC, CS
(Fux et al., 2005)	Europe	Switzerland	*In vitro*	19/29 (66)	CoNS	CVC, CS
(Mohamed Jamal et al., 2014)	North America	USA	CI - *In vitro*	5/5 (100)	MRSA, *S. epidermidis, E. coli, P. aeruginosa,* and *Candida albicans*	CS
(Souza et al., 2015)	South America	Brazil	CI - *In vitro*	4/4 (100)	*C. striatum* 1987/I, 2369/II, 1961/III and 1954/IV	CS
(Cherifi et al., 2014)	Europe	Belgium	*In vitro*	19/20 (95)	*S. epidermidis*	CVC
(Conlan et al., 2012)	North America	USA	*In vitro*	28/28 (100)	*S. epidermidis*	CVC, CS
(Percival et al., 2005)	Europe	UK	CI - *In vitro*	6/6 (100)	(MRSA, *S. epidermidis, E. coli, P. aeruginosa, C. albicans,* and *K. pneumoniae*	CS
(Petrelli et al., 2008)	Europe	Italy	*In vitro*	36/39 (92)	*S. aureus* and *S. epidermidis*	CVC
(Soumya et al., 2016)	Asia	India	*In vitro*	40/40 (100)	*S. epidermidis, S. haemolyticus, S. saprophyticus,* and *S. hominis*	CS
(Pereira et al., 2014).	South America	Brazil	*In vitro*	37/40 (93)	*S. haemolyticus*	CS
(S. Zhou et al., 2013)	Asia	China	*In vitro*	15/22 (68)	*S. epidermidis*	CS
(Mekni et al., 2012)	Africa	Tunisia	*In vitro*	97/97 (100)	*S. epidermidis*	CVC, CS
(Donelli et al., 2001)	Europe	Italy	CI - *In vitro*	74/97 (76)	*S. epidermidis* and *S. aureus*	CVC
(Tekeli, 2021)	Asia	Turkey	*In vitro*	35/35 (100)	*S. epidermidis* RP62A, *S. haemolyticus, S. hominis,* and *S. capitis*	CVC
(Olejnickova et al., 2014)	Europe	Czech Republic	*In vitro*	149/175 (85)	*P. aeruginosa*	CVC
(Kocianova et al., 2005)	North America	USA	MMCI - *In vivo*	N/A	*S. epidermidis* and *S. aureus*	CS

CI - *In vitro*: Catheter-infected *in vitro*; MMCI - *In vivo*: Mouse model of catheter infection – *In vivo*; MDR: Multidrug-resistant; MDS: Multidrug-susceptible; MRSA: Methicillin-resistant *Staphylococcus aureus*; CoNS: Coagulase-negative staphylococci; CS: Clinical sites; C: Catheter; CVC: Central venous catheters; UAC: Umbilical arterial catheter; UVC: Umbilical venous catheter; MD: Medical devices; N/A: Not Available.

strong biofilm formers (Sohail & Latif, 2018). Cherifi and colleagues demonstrated that 90% of *Staphylococcus epidermidis* isolates (18/20) were strong biofilm formers (Cherifi et al., 2014), while Petrelli and colleagues evidenced that 92% of *Staphylococcus aureus*, MRSA, and *Staphylococcus epidermidis* isolates were strong biofilm formers (36/39) (Petrelli et al., 2008).

Catheter-infected in vitro

An alternative approach to evaluating the ability to form biofilms is through catheter-infected *in vitro* assays (Fig. 3). In this approach, clinical isolates were previously collected from different biological samples or medical devices and then cultured into well containing medium and a catheter to analyze the biofilm production (Souza et al., 2015).

As shown in Table 5, most studies evaluated the biofilm production on polyurethane 16-gauge percutaneous nephrostomy catheters, whereas Brazil was the country with more studies of catheter-infected *in vitro*.

One of the most relevant results from infecting catheters *in vitro* was the influence of temperature on biofilm formation in abiotic surfaces (such as medical devices) evidencing that most strains showed lower biofilm production at 20°C when compared with the human body temperature at 37°C (Souza et al., 2015). When applying microscopic methodologies, the confocal analysis showed uniform colonization on uncoated catheters with *S. epidermidis* biofilms (Burton et al., 2006), while Scanning Electron Microscopy (SEM) showed microcolony formation of *Corynebacterium striatum* (Ramos et al., 2019) evidencing a large amount of mature biofilm and microcolonies of *C. striatum* in the surface of CVCs (Souza et al., 2015). To prevent the biofilm formation on

Table 2
General information on catheterized patients with nosocomial infections.

Hospital	Unit	Country	Nosocomial infection rate, n (%)	Male:Female	Age range	Main reasons for catheterization	Antibiotics during catheterization	Study
University Children's Hospital	NICU	Poland	15/40 (37.5)	27:13	23 to 41 weeks	Prematurity, therapeutic hypothermia monitoring, congenital disorders, and meconium aspiration syndrome	Yes	(Sobczak et al., 2019)
N/A	N/A	Pakistan	203/344 (59.0)	129:74	10 to 60 years	N/A	N/A	(Sohail & Latif, 2018)
Nationwide Children's Hospital	ICU	USA	16/22 (72.7)	14:8	1 to 14 years	N/A	Yes	(Jain et al., 2016)
Hospital Materno-Infantil Cochapamba	ICU	Bolivia	29/32 (90.6)	15:14	1 month to 5 years	Pneumonia, septicemia, and meningitis	Yes	(Cerezales et al., 2018)
University Hospital of Vern	Nephrology	Switzerland	17/26 (65.3)	18:8	29 to 83 years	Dialysis	Yes	(Fux et al., 2005)
Erasme University Hospital	ICU, nephrology, gastroenterology, neurology	Belgium	20/128 (15.6)	N/A	N/A	N/A	N/A	(Cherifi et al., 2014)
A teaching hospital in Rio de Janeiro	NICU	Brazil	21/40 (53.0)	N/A	0 to 28 days	N/A	Yes	(Pereira et al., 2014)
St. Anne's University Hospital	N/A	Czech Republic	149/172 (85.0)	104:71	0 to 100 years	N/A	N/A	(Olejnickova et al., 2014)

NICU: Neonatal Intensive Care Unit; ICU: Intensive Care Unit; N/A: Not Available.

Table 3
Analysis of biofilm-forming for different geographical regions.

Region	Number of studies	Biofilm rate	Prevalence of biofilms (%)
Europe	7	318/381	83.5
Asia	6	356/543	65.6
South America	6	55/59	93.2
North America	5	57/63	90.5
Africa	1	97/97	100

medical devices, Percival and colleagues applied treatment with tetra-sodium EDTA on catheters reducing *C. albicans* and MRSA biofilms after 21h, but with an additional 4h treatment, it was possible to eradicate these resistant microorganisms (Percival et al., 2005).

In vivo studies

The development of new strategies by coating CVCs to mitigate the production of biofilms has been considered a novel approach to combat infections (Hoque et al., 2016). Also, the application of animal models to reach the impact of coating CVCs allowed us to evaluate the mechanisms
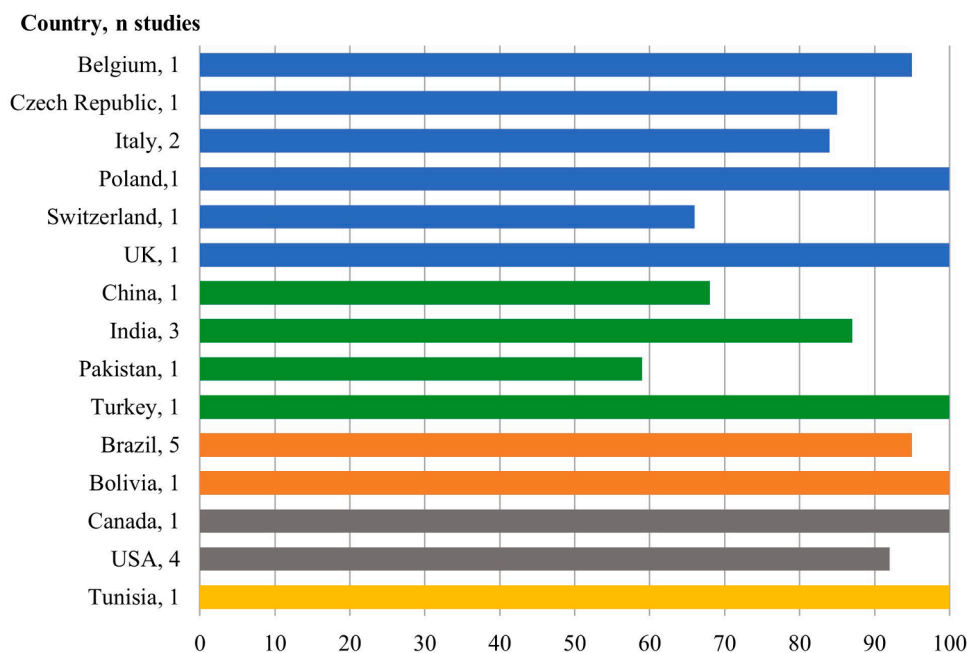

Fig. 2. Prevalence of biofilm formation in different countries.

Table 4

Biofilm-forming *in vitro* tests.

Methodology to measure biofilm	Biofilm classification	Country	Study
CV (595nm)	Weak (0.1> BFI ≤ 0.5), moderate (0.5> BFI ≤ 1), and strong (BFI > 1)	Pakistan	(Sohail & Latif, 2018)
CV (595nm)	N/A	USA	(Jain et al., 2016)
Test for slime production	N/A	India	(Sharma et al., 2011)
CV (620nm)	Weak (0.1> BFI ≤ 0.5), moderate (0.5> BFI ≤ 1), and strong (BFI > 1)	Bolivia	(Cerezales et al., 2018)
CV (540 nm)	Weak (BFI ≤ 1.5), moderate (1.5> BFI ≤ 3), and strong (BFI > 3)	Belgium	(Cherifi et al., 2014)
Safranin (490nm)	N/A	USA	(Conlan et al., 2012)
CV	Weak (BFI ≤ 0.120), moderate (0.120> BFI ≤ 0.240), and strong (BFI > 0.240)	Italy	(Petrelli et al., 2008)
QBA	N/A	India	(Soumya et al., 2016)
QD	N/A	Brazil	(Pereira et al., 2014)
QD	Color reference scale	China	(S. Zhou et al., 2013)
CV (490 nm)	The BU of negative control was used to classify biofilm production	Tunisia	(Mekni et al., 2012)
SEM	N/A	Italy	(Donelli et al., 2001)
N/A	N/A	Turkey	(Tekeli, 2021)
CV (595nm)	N/A	Czech Republic	(Olejnickova et al., 2014)

CV: Crystal violet; BFI: Biofilm formation index; QD: Qualitive Detection; QBA: Quantitative Biofilm Analysis; BU: Biofilm Unit; N/A: Not Available.

between the microorganism and the CVC in a host (Fig. 4) (Hoque et al., 2016; Kocianova et al., 2005). Catheter coated with polymer 7.5µg/mm^2 implanted in mice with ~1.7 × 10^7 CFU of MRSA showed a ~5 log reduction and revealed no cell clusters after 96h on the surface by SEM analysis (Hoque et al., 2016). Regarding bacterial pathogenesis,

S. epidermidis secretes poly-γ-DL-glutamic acid (PGA) allowing the colonization in a host, as approved by Kocianova and colleagues using a mice model infected with a PGA mutant *S. epidermidis* (Kocianova et al., 2005).

Biofilm-forming capacity according to different microorganisms

Gram-positive and negative bacteria and yeasts are well-known biofilm formers, being *Staphylococcus* spp*., E. coli, P. aeruginosa, K. pneumoniae,* and *S. epidermidis* the microorganisms more frequently reported in the literature (Muhsin Jamal et al., 2018). These microorganisms represent approximately 60% of all catheter biofilm-related infections. Moreover, *P. aeruginosa* has been used during the last decades as an *in vitro* model due to its ability to form biofilms (Cerezales et al., 2018; Khatoon et al., 2018). In this review, we summarized the biofilm-forming capacity of the microorganisms evaluated in our study set, as shown in Table 6.

Regarding the results, most of the strains in our study set showed that 13 of 18 microorganisms are 100% biofilm-formers, including several Gram-positive and negative bacteria as well as *Candida albicans*. Although the studies evaluated the ability to form biofilms under different laboratory-controlled conditions, all isolates are related to acquired-hospital infections, where *S. epidermidis* was isolated in 13 studies due to its high prevalence among patients with biofilm-associated infections (Cherifi et al., 2014). In summary, almost all staphylococcal species (such as *S. epidermidis, S. saprophyticus, S. capitis, S. cohnii, S. hominis, S. aureus,* and *S. haemolyticus*) showed a prevalence of biofilm-associated infections more than 90% in our study set. Meanwhile, MRSA evidenced a 60.8% of biofilm prevalence through four studies compiling a total of 360 isolates. Furthermore, all Gram-negative bacteria (*E. coli, P. aeruginosa, K. pneumoniae,* and *P. mirabilis*) reached 100% of biofilm prevalence except for *A. baumannii* evidencing 77.8% biofilm prevalence obtained in only one study. Finally, *C. albicans* and *C. striatum* also demonstrated a 100% of biofilm prevalence, being the only yeast and Gram-positive bacillus in the present review.

Biofilm and adhesion-related genes

The most prevalent biofilm-related infections are with *S. aureus, S. epidermidis*, and *E. coli* species in our study set. In staphylococcal species,
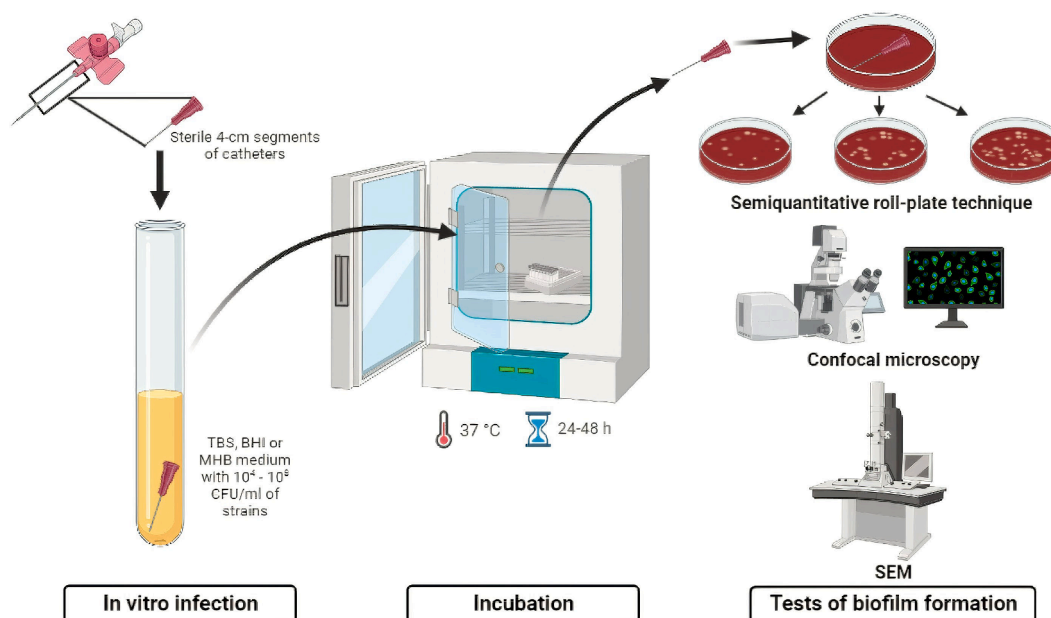

Fig. 3. Illustration of the procedure by catheter *in vitro* infection model.

Table 5
Biofilm-forming in catheter-infected *in vitro*.

Catheters type	Catheter-infected *in vitro*	Tests of biofilm formation	Country	Study
Polyurethane 16-gauge percutaneous nephrostomy catheters	In TSB medium containing 10^8 CFU/mL of strains	Viability of sessile forms of biofilm	Brazil	(Souza et al., 2019)
Coated silicon catheters	In BHI medium containing 10^7 CFU/mL of strains	Confocal microscopy	Canada	(Burton et al., 2006)
16-gauge percutaneous nephrostomy polyurethane and silicone catheters	In TSB medium containing 10^6 CFU/mL of strains	Semi-quantitative roll plate method and SEM	Brazil	(Ramos et al., 2019)
Polyurethane 16-gauge percutaneous nephrostomy catheters	In TSB medium containing 10^6 CFU/mL of strains	Semi-quantitative roll plate method and SEM	Brazil	(Sued et al., 2017)
Polyurethane CVC coated with CHX-M/R and uncoated catheter	In MHB medium containing 5×10^5 CFU/mL of strains	Counts for colony growth	USA	(Muhsin Jamal et al., 2018)
Polyurethane 16-gauge percutaneous nephrostomy catheters	In TSB medium containing 5×10^6 CFU/mL of strains	Quantitative and semi-quantitative roll plate methods and SEM	Brazil	(Souza et al., 2015)
Triple-lumen catheter	In TSB medium containing 10^4 CFU/mL of strains	Counts for colony growth and SEM	UK	(Percival et al., 2005)

SEM: Scanning Electron Microscopy; CFU: Colony-Forming Unit.

several genes are involved in biofilm production being the *ica* gene locus extensively studied. The *ica* gene locus regulates the biosynthesis of polysaccharide intercellular adhesion, which plays an important role in biofilm production. In addition, *mecA* genes are involved in methicillin resistance and are allegedly involved in the resistance patterns as well as biofilm induction (Mekni et al., 2012; Pereira et al., 2014). However, the relationship between these genes is not well-known. It is important to mentation that not all *ica*-positive isolates are biofilm producers, where Mekni and colleagues demonstrated in a previous study that only 43.9% of *S. epidermidis* with this cluster were able to produce biofilms (Mekni et al., 2012). Usually, *icaA* and *mecA* genes are present in most of the

Table 6
Prevalence of biofilms in the different microorganisms.

Species	Number of studies	Biofilm rate	Prevalence of biofilms (%)
Gram-positive bacteria			
Staphylococcus aureus	3	54/54	100
Staphylococcus haemolyticus	3	111/112	99.1
Staphylococcus epidermidis	12	416/416	100
Staphylococcus capitis	1	2/2	100
Staphylococcus cohnii	1	15/15	100
Staphylococcus saprophyticus	1	5/5	100
Staphylococcus hominis	2	48/48	100
MRSA	4	219/360	60.8
Coagulase-negative staphylococci	4	81/120	67.5
Corynebacterium striatum	3	10/10	100
Gram-negative bacteria			
Escherichia coli	4	4/4	100
Enterococcus faecalis	1	1/1	100
Acinetobacter baumannii	2	21/24	87.5
Klebsiella pneumoniae	3	3/3	100
Proteus mirabilis	1	1/1	100
Pseudomonas aeruginosa	4	178/178	100
Yeast			
Candida albicans	2	2/2	100

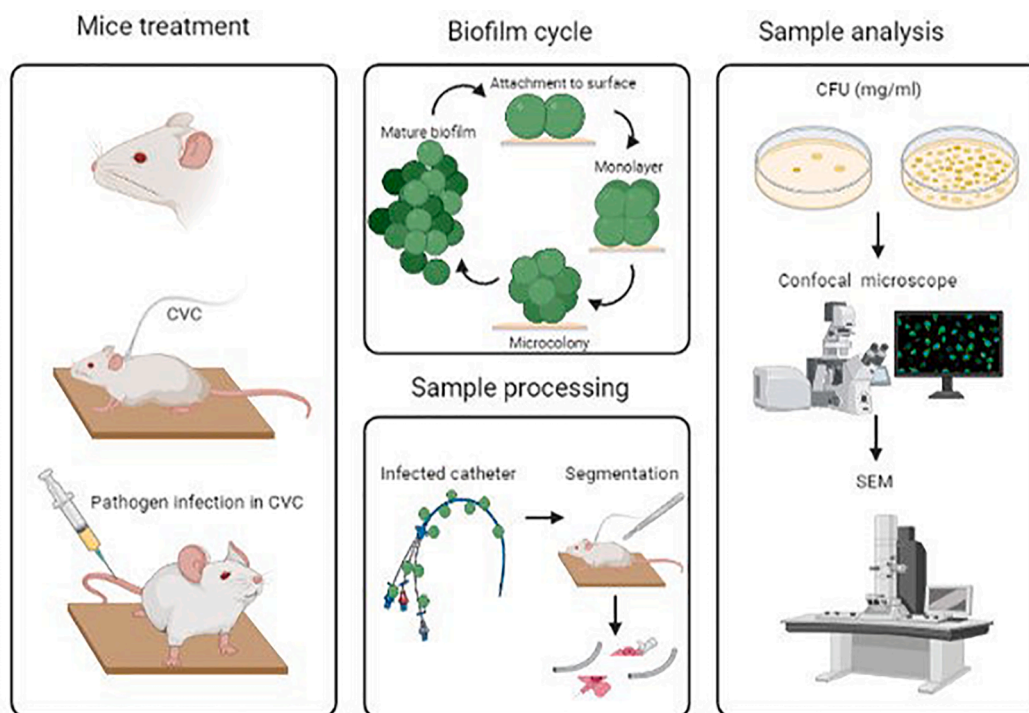MRSA: Methicillin-resistant *Staphylococcus aureus*

Fig. 4. Illustration of the procedure by catheter *in vivo* infection model.

S. aureus strains, resulting in MRSA evolution, but the presence of both genes does not indicate that a certain strain can form biofilm (Conlan et al., 2012; Donelli et al., 2001; Sharma et al., 2011). Therefore, further studies should be conducted to understand the multifactorial pathways leading to the establishment of biofilm among staphylococcal species.

On the other hand, *P. aeruginosa* biofilms have also been studied in the last two decades and it is an excellent biofilm producer, showing 100% of biofilm prevalence and being at the top of bacteria Gram-negative in the review. It is well-known the resilience of the mature *P. aeruginosa* biofilm when compared to the planktonic cells (Olejnick-ova et al., 2014). Nonetheless, the remaining Gram-negative bacteria are also important in nosocomial infections, in particular, *Acinetobacter baumannii* can be a serial problem in hospitals, because isolates are multidrug-resistant (MDR) and/or extensively drug-resistant (XDR), resulting in a high rate of mortality for patients. *A. baumannii* biofilm demonstrates an excellent evasion mechanism for infections and its huge antibacterial spectrum increases the complications of the patient infections and outcomes (Cerezales et al., 2018; Muhsin Jamal et al., 2018).

Prevention of biofilms on catheters

Several antibiofilm agents, such as antimicrobial CVCs, antimicrobial locks, enzymes, and polymers, have been tested with novel effects on different MDR pathogens and isolates from nosocomial infections, demonstrating significant inhibition and eradication rates against biofilms, where CVC coated with chitin derivates inhibited the biofilm formation of MRSA and CVC coated with chlorhexidine, minocycline, and rifampin showed a 100% of biofilm inhibition (Burton et al., 2006; Hoque et al., 2016; Mohamed Jamal et al., 2014; Percival et al., 2005). To evaluate the effects of different treatments after coating CVC, SEM analysis usually represents an elementary tool. As shown in Table 7, Percival and colleagues evaluated CVC coated with tetrasodium ethyl-enediamine tetraacetic acid (EDTA) at 40mg/mL reporting an eradication of 4 from 6 pathogens after 21h (Percival et al., 2005). On the other hand, MRSA and *C. albicans* biofilms were eradicated on CVCs coated with a certain polymer derivate from chitin at 7.5 $\mu g/mm^2$ as reported by Hoque and colleagues through SEM analysis (Hoque et al., 2016).

Likewise, when Jamal and colleagues coated CVC with chlorhexidine, minocycline, and rifampin (CHX-M/R), this antibiofilm combination proved to be effective against MRSA, *P. aeruginosa, C. albicans,* and *S. epidermidis* (Muhsin Jamal et al., 2018). Finally, *N,N'*-(1,2-phenylene) dimaleimide plus protamine sulfate (oPDM-plus-PS) at 50 mg/mL also inhibited the biofilm formation in 2 of the 6 isolates until 70% in *P. aeruginosa and S. epidermidis* (Burton et al., 2006). However, few studies were realized until now on CVCs with antibiofilm agents and further evaluation is needed.

Discussion

The importance of this systematic review lies in the fact that the high prevalence of biofilm formation on insertion medical devices, such as CVCs, dramatically increases the incidence of nosocomial infections in catheterized patients. Biofilms can usually be found on CVCs and most of them invaded the inside of the catheter depending on the duration of catheterization and length of hospital stay, which eventually lead to bloodstream infections in patients (Lewis, 2001).

The care units with a higher prevalence of nosocomial infections are NICU (Pereira et al., 2014; Sobczak et al., 2019) and ICU (Cerezales et al., 2018; Cherifi et al., 2014; Jain et al., 2016). It is well-known that ICU and NICU patients are at serious risk of contracting healthcare-associated infections or any bloodstream infection (Atiencia et al., 2022; Johnson & Quach, 2017). All evaluated clinical isolates in this systematic review were collected from patients with nosocomial infections, where most of the patients received several antibiotic treatments without success when applied against biofilms extracted from

Table 7
Antibiofilm studies on catheters.

Antibiofilm on catheters assays	Antibiofilm agents	Reducing the biofilm formation	Country	Study
Confocal microscopy	oPDM-plus-PS	59.1% inhibition to *P. aeruginosa* and 64.4% to *S. epidermidis*	Canada	(Burton et al., 2006)
SEM	Polymer derivate from chitin	Polymer-coated catheter revealed a lesser number of bacteria, thus indicating no biofilm formation on the surface	India	(Hoque et al., 2016)
CFU of the segments	Chlorhexidine, minocycline, and rifampin	A significant difference was reported for MRSA, *P. aeruginosa*, and *C. albicans*, while a biofilm inhibition of 100% was found against *S. epidermidis*	USA	(Muhsin Jamal et al., 2018)
SEM	Tetrasodium EDTA	After treatment eradicated the biofilm of *S. epidermidis*, *P. aeruginosa, K. pneumoniae,* and *E. coli,* while also reducing MRSA biofilm by 3.5 logs and *C. albicans* biofilm by 2.2 logs CFU	UK	(Percival et al., 2005)

SEM: Scanning Electron Microscopy; CFU: Colony-Forming Unit.

them. This issue occurred because a routine systemic treatment with antibiotics is not effective due to resistance and tolerance of biofilm organisms present on these medical devices (Donlan, 2008; Galié et al., 2018; Rodríguez-Cerdeira et al., 2020; Roy et al., 2018). When comparing gender, all studies reported differences in the rate of nosocomial infections in catheterized patients between men and women, evidencing a higher susceptibility in men to develop this type of biofilm-associated infection, as recently postulated by Tomczyk-Warunek and colleagues(Tomczyk-Warunek et al., 2021).

All clinical isolates evaluated in the present review were directly isolated from catheters and/or biological samples/biopsies from catheterized patients. The procedure of the *in vitro* catheter infection model was originally formulated through intravenous catheter-associated infections in patients, which showed extreme microbial colonization and related to some particular diagnoses like severe sepsis, suppurative thrombophlebitis, endocarditis, bloodstream infection, and biofilm-related strains collected from the blood or even skin (Atiencia-Carrera et al., 2022a; Mermel et al., 2009; Pinto et al., 2021).

Concerning the geographical distribution of our study set, it is remarkable that Europe showed the highest number of published studies, but America and Africa demonstrated a higher prevalence of biofilm-forming strains. However, it is important to increase the number of studies on Asia and Africa. Only one study was published in Africa, while Asian studies reported a significantly lower prevalence of biofilm infections when compared with the remaining regions. Moreover, European countries have developed several problems related to microbial infections. In particular, Italy was reported as one of the worldwide regions with an increase in deaths due to antibiotic resistance and biofilm-related infections (Atiencia-Carrera et al., 2022a; Cesta et al., 2020; Nolan et al., 2020). Other regions also described the high

prevalence of nosocomial infections caused by catheterization such as Bolivia, where there has been a steady increase in the mortality rate related to nosocomial infections since 2001 (Cerezales et al., 2018; Maury Fernández et al., 2003), and Turkey, where the mortality rate associated with these infections reached values of 69% (Cevik et al., 2005; Dagi et al., 2016).

Several *in vitro* methods that have been developed to characterize the ability of clinical isolates in biofilm formation are usually based on colorimetric or microscopic techniques. Currently, crystal violet (CV) staining technique, due to its simplicity and sensitivity, is the preferred method for *in vitro* biofilm quantification and classification (Atiencia-Carrera et al., 2022b; Di Domenico et al., 2016; Stepanović et al., 2000). Another advantage of the CV staining technique, as a basic stain, is the ability to stain all living and dead cells by binding to the negative charge of surface molecules and extracellular matrix polysaccharides (Extremina et al., 2011). Although some studies found at least one biofilm-forming strain, most of the studies reported multispecies biofilms, where CV staining could be applied to either type of biofilm and classified them as strong, moderate, and weak biofilm formers. This classical classification is usually done through the BFI. The BFI is obtained through a mathematical algorithm and measures the adhesion capacity of each strain by comparing the initial and the final absorbance measurements during a certain incubation growth period (Atiencia-Carrera et al., 2022a; Castro et al., 2022; Olivares et al., 2016). This systematic review also describes studies using the procedure by catheter *in vitro* infection model. These studies allow predicting thebehavior of biofilm in medical devices, from initial adhesion until biofilm dispersion and even antimicrobial biofilm treatments in catheters with different and relevant clinical isolates (Asker et al., 2021; Buhmann et al., 2016; Didehdar et al., 2022). Due to the phenotypic shift associated with biofilms and the environmental and medical settings, this *in vitro* model allowed us to characterize altered gene expression, virulence factors, antimicrobial resistance mechanisms, and biofilm life cycle (Atiencia-Carrera et al., 2022b; Dötsch et al., 2012; Hall & Mah, 2017). Thus, most studies using CV staining technique also employ other methodologies to measure biofilm formation on catheters, such as Confocal Laser Scanning Microscopy (CLSM) and SEM. These techniques provide a high-resolution insight into the *in vitro* process of biofilm first attachment to the catheters' surface. Overall, SEM analysis showed microbial colonization on catheters in all studies where this *in vitro* infection model was applied in our study set. While CLSM analysis could evidence the three-dimensional colonization on catheter surfaces (Burton et al., 2006; Rosenberg et al., 2019).

The catheter *in vivo* infection model plays a relevant role in monitoring biofilm infections, giving a pathway to characterize omics, quorum sensing molecules, immune responses, treatment outcomes, and infection evolution (Chauhan et al., 2016; Su et al., 2020). In our study set, several researchers used *in vivo* model to assess the antibiofilm activity of various alternative and standard therapies against several MDR strains (like MRSA and vancomycin-resistant enterococci), such as coating CVCs with polydimethylsiloxane (PDMS) to reduce biofilm development. Hoque and colleagues achieved more than 84% of biofilm reduction in all MDR strains analyzed (Hoque et al., 2016). Likewise, Zhou and colleagues demonstrated similar results when employing polymers at 7.5 µg/mm^2 by coating CVCs. This type of coating showed novel antibiofilm and antimicrobial properties against MRSA and MDR *S. epidermidis* strains (C. Zhou et al., 2017). By SEM analysis, it was possible to confirm the conformation of the polymer agent on the CVC surface and the inhibition and eradication of biofilms. While dead/alive staining and CLSM evaluation allowed us to validate the mortality rate and biofilm composition (Chauhan et al., 2016; C. Zhou et al., 2017). The preliminary animal-model results lead to the initial evaluation to characterize further the interaction host-CVC-infection (Su et al., 2020).

Among the results obtained in our study set, most of the clinical isolates belong to *S. epidermidis*, regarding its importance in nosocomial infections and biofilm formation capacity. Although *S. epidermidis* was an underrated opportunistic pathogen, studies in the last decades became essential to clarify the importance of this species and understand its mechanisms, genes, antibiotic resistance, and biofilm ability (Le et al., 2019). *S. epidermidis* is nowadays characterized by its resistant intercellular network of amyloid fibers, which became an important factor during the biofilm cycle, increasing the resistance to environments, chemical factors, standard treatments, and several antibiotics (Yarawsky et al., 2020). On the other hand, MRSA is one of the major nosocomial pathogens known worldwide, in particular by the mechanisms of antibiotic resistance and biofilm formation, reaching a biofilm rate almost of 99%, being strong biofilm formers in more than 30% and 50% of them are isolated from catheters infection (Piechota et al., 2018; Suresh et al., 2019). Meanwhile, Gram-negative bacteria represent a significant percentage of nosocomial infections and potential to establish biofilm. Regarding the results obtained in the study set, more than 87% of Gram-negative isolates are biofilm producers, in agreement with the literature, being detected around 84% in CVC-related infections with *E. coli, K. pneumoniae, P. aeruginosa,* and *Acinetobacter* spp. Consequently, urge for new therapies are urgently needed to inhibit and eradicated biofilms in catheter-associated infections, as postulated by several authors (Gunardi et al., 2021; Maharjan et al., 2018; Oleksy-Wawrzyniak et al., 2021). Finally, *Candida* isolates equally demonstrated to be strong biofilm-formers, through their production of extracellular polysaccharides and hyphae morphology shift, showing a biofilm prevalence near 100% and so becoming an important threat in hospital-acquired infections (Bekkal brikci benhabib et al., 2021).

Nonetheless, controversies in the literature can be found about the relationship between biofilm formation and antibiotic resistance (Carcione et al., 2022; Ruhal & Kataria, 2021). Since not all *S. epidermidis* strains with a resistance spectrum are biofilm producers, it is believed that horizontal gene transfer (HGT) mechanisms also play a vital role in antimicrobial resistance in hospital-acquired infections (Abe et al., 2020). As postulated by Abe and colleagues, biofilms constitute a hot spot of HGT of antibiotic resistance genes, through conjugation, transformation, and transduction, in any environment like health care facilities (Abe et al., 2020). The same scenario was found in several studies with *S. aureus* isolates, where the correlation between biofilm production and antibiotic resistance is inconclusive. Indeed, the studies reported a higher percentage of biofilm formers, but not all of them are MRSA or meticilin-sensitive *Staphylococcus aureus* (MSSA), whereas sensible isolates also are biofilm positive. Moreover, *ica ABCD* operon is associated with biofilm production, where a polysaccharide intercellular adhesin is encoded, being an essential factor for the biofilm of *Staphylococcus* spp. However, the presence of the entire cluster did not always correlate with biofilm production, but there is evidence to suggest a correlation when at least two genes (*icaAD*) need to be co-transcribed. Therefore, the presence of *ica ABCD* operon does not necessarily determine the biofilm production among the strains (Abdel-Shafi et al., 2022; Kıvanç, 2018; Sharma et al., 2011). Finally, among Gram-negative isolates, the studies reported the same paradigm, appointing other potential mechanisms such as secretion of different polysaccharides, amyloid-type proteins, flagella, and virulence factors (Conlan et al., 2012; Senobar Tahaei et al., 2021; Wang et al., 2020). Although some studies evaluated novel compounds against *in vitro* biofilm formation by coating catheters with antimicrobials, polymers, and lock solutions (Chandra et al., 2018; Percival et al., 2005), further *in vitro* and *in vivo* studies are strictly necessary to discover and implement alternative treatments for biofilm inhibition and eradication.

Conclusions

In summary, this systematic review evaluated published studies on biofilm-forming microorganisms isolated from CVCs and analyzed the prevalence of biofilm among catheterized patients and their association with nosocomial infections. Although the reviewed studies employed different methodologies for the assessment of biofilm formation, an

accurate analysis was realized describing the most frequently isolated biofilm-forming species, their biofilm classification, and geographical distribution. The information included in this systematic review uses data published worldwide and without time delimitation. Finally, our systematic analysis showed that the high prevalence rate of biofilm-forming microorganisms is related to the high occurrence of nosocomial infections in catheterized patients. Further studies are needed to determine the real incidence of biofilms and their microbial diversity in countries for which results have not yet been reported, and a full report of the clinical background of the patients in published studies is essential to better understand the interaction between biofilm-associated infections and host immune response.

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Data availability statement

All data presented in this study are available upon request by contacting the corresponding author A.M.

CRediT authorship contribution statement

Sandra Pamela Cangui-Panchi: Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft. **Anahí Lizbeth Ñacato-Toapanta:** Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft. **Leonardo Joshué Enríquez-Martínez:** Conceptualization, Formal analysis, Investigation, Writing – original draft. **Jorge Reyes:** Conceptualization, Writing – review & editing. **Daniel Garzon-Chavez:** Conceptualization, Resources, Writing – review & editing, Funding acquisition. **António Machado:** Conceptualization, Methodology, Resources, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Data availability

Data will be made available on request.

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