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Antimicrobial resistance profiles of bacterial pathogens isolated from the bloodstream in patients of a hospital institution in Montería — Córdoba

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Abstract

Objectives: To determine the most frequent bacterial pathogens in the bloodstream and their resistance profiles in a hospital institution in Montería - Córdoba. **Methods:** An observational study was conducted, during the months of January to December 2018, 113 blood samples from patients with sepsis were evaluated. The identification and susceptibility of the bacteria were determined through the VITEK system, using the GNI + cards for Gram-negative bacilli and GPI for Gram-positive cocci and the GNS 113 and GPS 102 cards for Gram-negative bacilli and Gram-positive cocci respectively. **Findings:** Among the Gram-negative bacteria, the ones that were most frequently isolated were *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*; while among the Gram-positive bacteria *Staphylococcus epidermidis* was the pathogen that was most frequently isolated. Gram-negative *Pseudomonas aeruginosa* isolates showed high resistance to doripenem, imipenem and meropenem. *Acinetobacter baumannii* showed high resistance to most antibiotics. *Serratia marcescens*, *Escherichia coli*, *Enterobacter cloacae* had a low resistance rate. Gram-positive *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis* ss. *hominis*, *Enterococcus faecalis* and *Staphylococcus aureus* ss. *aureus* showed high resistance to erythromycin. **Novelty:** A high prevalence of antimicrobial resistance was observed in the present investigation.

Keywords: Blood culture; bacteremia; antimicrobial resistance; sepsis

1 Introduction

The presence of live microorganisms in a patient's blood is usually indicative of a serious invasive infection that requires urgent antimicrobial treatment^(1,2). Approximately 200,000 cases of bacteraemia occur annually worldwide with mortality rates ranging from 20% to 50%, which is why they require rapid and adequate empirical therapy before the blood culture results are ready; blood cultures are essential in the diagnosis and treatment of etiologic agents⁽³⁻⁵⁾.

The lethality of sepsis in terms of mortality rate has decreased over the past two decades, however, the increase in cases of sepsis, particularly in developing countries, remains a major health problem that creates a major challenge for clinicians in the selection of suitable antimicrobial agents, since it is further complicated by the increasing development of resistance of bacteria to antimicrobial agents, which is the pillar of sepsis treatment⁽⁶⁾. Both Gram-positive and Gram-negative bacteria have been isolated from the bloodstream and the predominance of one type over another varies from place to place⁽⁶⁾.

Various epidemiological studies of bloodstream infections (ITOS) have been carried out in Europe and the United States of America (USA), with patients registered in national databases; It is estimated that in Europe there are 1,213,460 to 1,381,590 episodes with 157,000 deaths per year and in the United States of America 575,462 and 677,389 with 79,000 to 94,000 deaths per year^(7,8). In the countries like South Africa and Ethiopia, the rate of Sepsis mortality is almost 53%, which is considered a major health problem in developing countries⁽⁹⁾. Being the most prevalent bacteria *Staphylococcus aureus*, *Staphylococcus negative coagulase*, *Enterococcus* spp., *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* y *Escherichia coli*^(10,11). In Latin America, although the same volume of information does not exist, epidemiological studies of bacteraemia have been carried out in national surveillance programs in Brazil (SCOPE) or from the international antimicrobial resistance surveillance program SENTRY⁽¹²⁾.

In Colombia there have been few epidemiological studies of sepsis, however there are studies published by Vásquez et al., 2018, who characterized pediatric patients with positive blood cultures from the pediatric intensive care service of the San José Bogotá Hospital, April 2012 to 2017, reporting the most frequent non-contaminating isolated germs such as *Staphylococcus aureus* (30%), followed by *Klebsiella pneumoniae* (17.5%) and *Streptococcus pneumoniae* (17.5%). The most frequent contaminating germ was *Staphylococcus epidermidis* (47.5%), also Cortes et al., 2013 determined the frequency of isolated microorganisms in patients with bacteraemia in intensive care units in Colombia and their resistance profiles, the microorganisms most frequently were *Staphylococcus aureus* 12.5%, *Klebsiella pneumoniae* 8.2%, *Escherichia coli* 5.7%, *Acinetobacter baumannii* y 4.0% and *Pseudomonas aeruginosa* 3.8%. Negative coagulase *Staphylococcus* recorded an oxacillin resistance rate greater than 70%. There was a trend towards a lower resistance rate among the isolates of *E. coli* y *K. pneumoniae* during the study period, while the carbapenem resistance rate of *A. baumannii* exceeded 50%.

In Montería (Córdoba), the first epidemiological study of sepsis and its resistance profiles has not been carried out, with the present investigation it is sought to provide updated information on the main bacteria isolated from the bloodstream and the tendency of antibiotic resistance profiles, serving as an aid to doctors in our region when choosing the most appropriate therapy. Because the resistance may be different according to the region and according to geographical and epidemiological characteristics.

2 Materials and Method

2.1 Study design

An observational prospective study was carried out in a clinic in the city of Montería - Córdoba, during the months of January to December 2018.

2.2 Sampling

During the study period, 113 blood samples from patients with sepsis were evaluated. Informed consent was obtained from patients or close relatives of patients before being included in the study. All patients of both sexes, all age groups with suspected or proven infection were admitted⁽⁶⁾.

Blood samples were collected by trained laboratory personnel at the doctor's request. Two blood samples were collected before patients were treated with antibiotics. Samples were collected after a thorough cleaning of the venous site with 70% alcohol and 2% iodine tincture. Under aseptic conditions, 10 ml of blood was drawn by venous puncture and about 5 ml of blood was inoculated into each of the 50 ml of tryptone soy broth and incubated at 37 ° C^(13,14). Turbidity (growth sign bacterial) was checked daily until day 14 to report that there was no bacterial growth. The turbid broth cultures were subcultured in MacConkey agar, blood agar, chocolate agar and salted mannitol agar, to be subsequently incubated at 37 ° C for 24 to 48 hours^(15,16). Subsequently, pure colonies were grown in agar nutritive and blood agar for identification through the VITEK system, using the GNI + cards for Gram-negative bacilli and GPI for Gram-positive bacilli. Prior to the inoculation of the cards, Gram staining and oxidase, catalase and / or coagulase reaction according to the microorganism were carried out in all cases⁽¹⁷⁾.

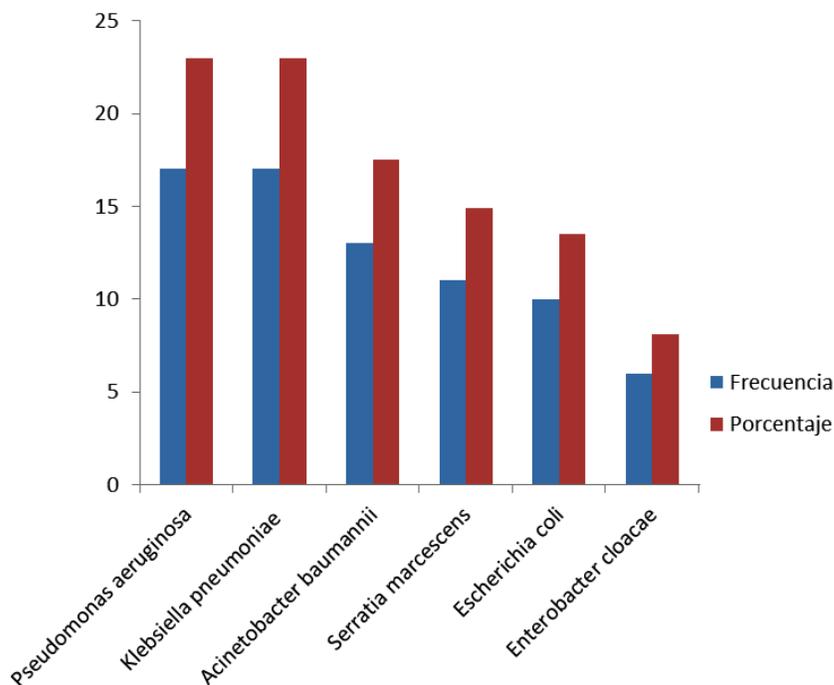


Fig 2. Frequency of occurrence of Gram - negative microbial strains responsible for sepsis.

The most frequently identified Gram-positive bacteria were the *Staphylococcus epidermidis* 9 (29.2%), *Staphylococcus haemolyticus* 6 (19.3%), *Staphylococcus hominis* ss. *Hominis* 6 (19.3%), *Enterococcus faecalis* 5 (16.1%) and *Staphylococcus aureus* ss. *Aureus* 5 (16.1) (Figure 3).

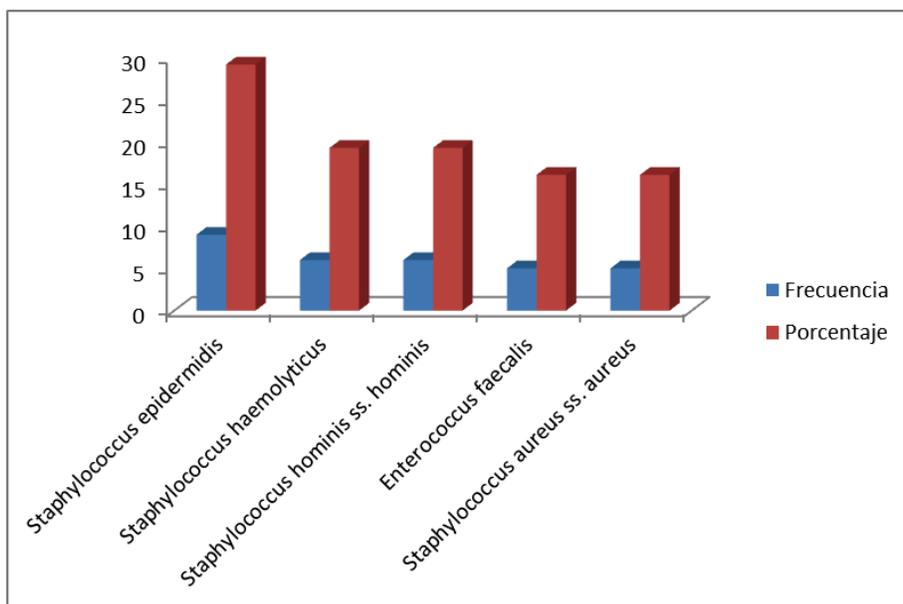


Fig 3. Frequency of occurrence of Gram - positive microbial strains responsible for sepsis

3.3 Resistance of microorganisms Gram — negative to antimicrobial agents

The sensitivity and resistance of the Gram-negative bacteria are shown in Table 1. The antimicrobials used were piperacillin-tazobactam, ceftazidime, cefepime, doripenem, imipenem, meropenem, amiKacina, gentamicin, ciprofloxacin and Colistin. *Pseudomonas aeruginosa* has a greater resistance to doripenem (50%) and imipenem (56.2%); *Klebsiella pneumoniae* showed resistance to ceftazidime (25%) and cefepime (25%); *Acinetobacter baumannii* showed resistance to most drugs such as piperacillin-tazobactam (61.9), ceftazidime (61.9), cefepime (61.9), doripenem (61.9), imipenem (61.9), meropenem (61.9) and ciprofloxacin (61.9); *Serratia marcescens* presented a low resistance to ceftazidime (11,1); *Escherichia coli* showed resistance to ceftazidime (52.4) and cefepime (52.4); *Enterobacter cloacae* presented a low percentage of resistance to most of the antimicrobials used piperacillin-tazobactam (8.4%), ceftazidime (8.4%), cefepime (8.4%), doripenem (8.4%), imipenem (8.4%), meropenem (8.4%), ciprofloxacin (8.4%) and colistin (8.4%).

Table 1. Antibiotic susceptibility pattern of Gram-negative bacteria isolated from blood cultures

Isolation		PIP-TZ N/ (%)	CAZ N/ (%)	FEP N/ (%)	DPM N/ (%)	IPM N/ (%)	MEM N/ (%)	AMK N/ (%)	G N/ (%)	CIP N/ (%)	COL N/ (%)
<i>Pseudomona saeruginosa</i> (18)	S	9 (50)	10 (56,2)	10 (56,2)	9 (50)	8 (43,8)	9 (50)	12 (68,8)	15 (81,2)	10 (56,2)	100
	I	2 (12,5)	2 (12,5)	2 (12,5)	0 (0)	0 (0)	1 (6,2)	2 (12,5)	0 (0)	0 (0)	0 (0)
	R	7 (37,5)	6 (31,2)	6 (31,2)	9 (50)	10 (56,2)	8 (43,8)	4 (18,8)	3 (18,8)	8 (43,8)	0 (0)
<i>Klebsiella p neumoniae</i> (18)	S	15 (81,2)	14 (75)	14 (75)	17 (93,8)	17 (93,8)	17 (93,8)	17 (93,8)	17 (93,8)	14 (75)	
	I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0	1 (6,2)	1 (6,2)	
	R	3 (18,8)	4 (25)	4 (25)	1 (6,2)	1 (6,2)	1 (6,2)	1 (6,2)	0 (0)	3 (18,8)	
<i>Acinetobacter baumannii</i> (18)	S	7 (38,1)	4 (23,8)	7 (38,1)	7 (38,1)	7 (38,1)	7 (38,1)		7 (38,1)	7 (38,1)	15 (85,7)
	I	0 (0)	3 (14,3)	0 (0)	0 (0)	0 (0)	0 (0)		6 (33,3)	0 (0)	0 (0)
	R	11 (61,9)	11 (61,9)	11 (61,9)	11 (61,9)	11 (61,9)	11 (61,9)		5 (28,6)	11 (61,9)	3 (14,3)
<i>Serratiamarcescens</i> (12)	S		9 (70,4)	12 (100)	12 (100)		12 (100)	12 (100)	12 (100)	12 (100)	
	I		2 (18,5)	0 (0)	0 (0)		0 (0)	0 (0)	0 (0)	0 (0)	
	R		1 (11,1)	0 (0)	0 (0)		0 (0)	0 (0)	0 (0)	0 (0)	
<i>Escherichia coli</i> (10)	S	8 (84,2)	4 (47,6)	4 (47,6)	8 (84,2)	8 (84,2)	8 (85)	10 (100)	10 (100)	3 (33)	8 (84,2)
	I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (15,8)
	R	2 (15,8)	6 (52,4)	6 (52,4)	2 (15,8)	2 (15,8)	2 (15)	0 (0)	0 (0)	7 (66,7)	0 (0)
<i>Enterobacter cloacae</i> (6)	S	4 (83,3)	3 (66,6)	5 (91,7)	5 (91,7)	5 (91,7)	5 (91,7)	6 (100)	5 (91,7)	3 (66,7)	3 (66,7)
	I	1 (8,3)	2 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (8,3)	2 (25)	2 (25)
	R	1 (8,4)	1 (8,4)	1 (8,3)	1 (8,3)	1 (8,3)	1 (8,3)	0 (0)	0 (0)	1 (8,3)	1 (8,3)

N:number of sensitive isolates

Piperacillin-Tazobactam (PIP-TZ); ceftazidime (CAZ); Cefepime(FEP); Doripenem (DPM); Imipenem (IPM); Meropenem (MEM); AmiKacina (AMK);Gentamicin (G); Ciprofloxacin (CIP); Colistin (COL)

3.4 Resistance of Gram-positive microorganisms to antimicrobial agents

The sensitivity and resistance of Gram-positive bacteria are shown in Table 2. The antimicrobials used were trimethoprim/sulfamethoxazole, erythromycin, nitrofurantoin, ciprofloxacin, levofloxacin, vancomycin, gentamicin, teicoplanin, minocycline, rifampin and tetracycline. Of the antimicrobials used *Staphylococcus epidermidis* showed resistance to erythromycin (13%) and ciprofloxacin (18.2%); *Staphylococcus haemolyticus* showed no resistance to any of the antibiotics used; *Staphylococcus hominis ss. hominis* presented resistance to erythromycin (40%) and nitrofurantoin (40%); *Enterococcus faecalis* and *Staphylococcus aureus ss. aureus* showed resistance to erythromycin (40%).

Table 2. Antibiotic susceptibility pattern of Gram-positive bacteria isolated from blood cultures.

		TMP/SMX	E	NIT	CIP	LFX	VAN	GEN	TEC	MINO	RIF	TET
<i>Staphylococcus epidermidis</i> (9)	S	8 (85,7)	1 (6,7)	7 (81,8)	6 (72,7)	7 (85,7)	9 (100)	9 (100)	9 (100)	9 (100)	9 (100)	9 (100)
	I	0 (0)	2 (13,3)	1 (9,1)	2 (18,2)	1 (7,1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	1 (14,3)	6 (80)	1 (9,1)	1 (9,1)	1 (7,1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Staphylococcus haemolyticus</i> (6)	S	6 (100)	6 (100)	6 (100)	5 (83)	5 (83)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)
	I	0 (0)	0 (0)	0 (0)	1 (17)	1 (17)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Staphylococcus hominis ss. hominis</i> (6)	S	5 (83)	3 (60)	3 (60)	(6) 100	(6) 100	(6) 100	(6) 100	(6) 100	(6) 100	(6) 100	(6) 100
	I	1 (17)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	2 (40)	2 (40)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Enterococcus faecalis</i> (5)	S	5 (100)	3 (60)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)
	I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	2 (40)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Staphylococcus aureus</i> (5)	S	5 (100)	3 (60)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)
	I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	2 (40)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

N: number of sensitive isolates

Trimethoprim / Sulfamethoxazole (TMP / SMX);Erythromycin (E); Nitrofurantoin (NIT); Ciprofloxacin (CIP); Levofloxacin(Lfx); Vancomycin (VAN); Gentamicin (GEN); Teicoplanin (TEC); Minocycline(MINO); Rifampicin (RIF); Tetracillin

4 Discussion

Given the increasing resistance of bacteria to antimicrobials, surveillance programs have become important in defining the distribution of species and the resistance patterns of pathogens that cause bloodstream infections, therefore, they are the basis for adequate empirical therapy⁽¹⁸⁾. Bacterial infection of the bloodstream is one of the main agents that cause morbidity and mortality throughout the world, which requires urgent and effective treatment to control infections⁽⁴⁾; because mortality rates double from 30% to 60%, when inadequate empirical treatment was administered to patients in the Intensive Care Unit (ICU) with bloodstream infections⁽¹⁹⁾. In this prospective study, information is provided on the distribution of bacterial isolates that cause infections in the bloodstream along with their antibiotic susceptibility pattern, crucial information in the effective management of septicemic cases.

According to Biedenbach et al., 2004, the frequency of occurrence of bacterial species that cause bloodstream infections in different parts of the world was quite variable; demonstrating that Gram-positive species were causative agents in 57% of infections in the Medical Centers of North America compared to a minority of cases in Latin America and Europe (51%). However, Orsini et al., 2012 and Wisplinghoff et al., 2004 reported that in the United States of America there is a tendency towards an increase in the incidence of Gram-negative organisms that cause infections in the bloodstream. This variation of the etiologic agents from one country to another could be due to geographical locations, epidemiological differences in the

etiological agents, other factors may also be due to the nature of the patient population, the limited sample size and the study period. In the present investigation, Gram-negative bacteria (n = 82, 72.6%) were the most frequent cause of bloodstream infections (Figure 1); Similar results were reported studies conducted by Droz et al., 2019, in the Netherlands on pathogenic bacteria in the bloodstream in pediatrics also reported a greater number of isolated Gram-negative bacteria (63.9%), compared to Gram-positive (35.8%) in Colombia by De la Rosa et al., 2016, which conducted a prospective multicenter study, conducted in ten hospitals in four cities between September 2007 and February 2008, similar results have been reported in Mexico by Sánchez et al., 2010; in Argentina by Saad et al., 2018, in Iran by Rabirad et al., 2014 and in Nepal by Prakash et al., 2016. The majority of cases of Gram-negative bacteraemia that occurred in hospitalized patients were associated with healthcare as is patient management and hand washing^(20–24).

Among the Gram-negative bacteria, the ones that were most frequently isolated were *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* (Figure 2), the reason for the high isolation rates of these Gram-negative bacteria may be due to the acquisition of an infection during the hospital stay, since they are recognized opportunistic pathogens, which mainly affects hospitalized patients and also remains on inanimate surfaces for long periods, this fact facilitates its dissemination in the hospital environment and the hands of health personnel constitute a fundamental vehicle of transmission in the frequent occurrence of outbreaks, the incidence of infections has continuously increased in the last two decades^(25,26). Our results are consistent with another study with *Pseudomonas* spp. It was the most common bacterial organism that caused bloodstream infections.

Gram-positive bacteria also represent a serious threat because their morbidity in bloodstream infections is constantly increasing worldwide^(27,28). Among Gram-positive bacteria, *S. epidermidis* was the pathogen that was most frequently isolated in the present study, followed by *S. haemolyticus* (figure 3). These two species have been reported in national and international studies as the most frequent among nosocomial infections⁽²⁹⁾. De la Rosas et al., 2016 also reports *S. epidermidis* as the Gram-positive bacterium most frequently in isolation of bloodstream infections, it was also reported by Cortes et al., 2017 and Mamishi et al., 2005. *S. epidermidis* it was recognized as low incidence pollutants until the 1970s; however, several studies have reported an increase in the incidence of infection with this bacterium⁽³⁰⁾, which are of growing concern, due to the high distribution of methicillin resistance among the isolates and their durability in the devices, which it often results in the replacement of the device, which causes more trauma and cost⁽²⁶⁾. From the epidemiological point of view, he has developed strategies to conquer the hospital environment, such as his ability to colonize the inert surface of invasive medical devices and forming biofilms. The formation of biofilms in *S. haemolyticus* has not yet been fully clarified⁽²⁹⁾.

As published by the World Health Organization in the United States, antimicrobial resistant microorganisms (AMR) cause more than 2 million infections and are associated with approximately 23,000 deaths each year and the European Center for the Prevention and Control of Diseases (ECDC) reported that AMR is associated with approximately 25,000 deaths per year^(31,32). In the present study, the antimicrobial resistance profile of Gram-negative bacteria showed a higher resistance rate compared to Gram-positive bacteria, such as it has also been demonstrated in other studies^(33,34). *Pseudomonas aeruginosa* showed high sensitivity to most of the drugs used, however it showed high resistance to doripenem (50), imipenem (56.2) and meropenem (43.8). Comparing the results obtained with another study conducted in Latin America (Argentina, Brazil, Chile, Costa Rica, Ecuador, Guatemala, Mexico, Panama, Peru and Venezuela, Colombia), it was possible to observe a similarity in the results with a resistance to imipenem (44.9%), meropenem (38.4%) and doripenem (49%)^(35–37). This may be due to empirical and inappropriate use as a first-line treatment. This is an alarming sign for clinicians because it leaves a very limited choice of medications such as colistin and tigecycline, which have serious side effects and toxicity⁽³³⁾. While *Klebsiella pneumoniae* showed high sensitivity to all antibiotics used in ranges between 75% - 93.8%, similar results were reported by Alam et al., 2011.

Acinetobacter baumannii isolates showed high resistance to most antibiotics with a percentage of 61.9%, with the exception of colistin which presented a resistance of 14.3%. According to Cortes et al., 2013 in Colombia, a five-fold increase in resistance was reported between 2001 and 2008. Globally, this bacterium has also exhibited high resistance to most antibiotics⁽³⁵⁾. There are multiple studies that explore the treatment alternatives for *A. baumannii* infection, antibiotic therapies that have been ruled out because of high toxicity rates, such as colistin, have been resumed, these antibiotics have shown efficacy in the treatment of multidrug-resistant strains of this microorganism⁽¹⁴⁾. While the rates of resistance to multiple drugs in isolates of *Serratia marcescens*, *Escherichia coli*, *Enterobacter cloacae* were low; similar studies have been reported as those conducted by⁽⁹⁾.

S. epidermidis y *S. haemolyticus* they are frequent natural colonizers of the wet surfaces of the body, such as the armpits, the inguinal area and the perineal area; they have adapted to become nosocomial pathogens because they exhibit resistance to antibiotics and antiseptics, as well as their ability to produce biofilms. In the present work *S. epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis* ss. *hominis*, *Enterococcus faecalis* y *Staphylococcus aureus* ss. *Aureus* showed high resistance to erythromycin (Table 2). Similar results were published by⁽³⁸⁾ where they reported a high resistance of these bacteria to erythromycin (62% of isolates), oxacillin (51%), gentamicin (61%), lincomycin (60%), ofloxacin (60%) and rifampicin (51%).

Bora et al., 2018 also reported resistance to several non- β -lactam antimicrobial types such as ciprofloxacin (58% –72%), gentamicin (26% –72%) and cotrimoxazole (46% –47%).

5 Conclusion

A high prevalence of antimicrobial resistance was observed in the present investigation, particularly in Gram-negative bacteria. This high prevalence of antimicrobial resistance rates in the region may be due to excessive drug use due to its easy availability.

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