

Multidrug-resistant bacteria isolated from surgical site of dogs, surgeon's hands and operating room in a veterinary teaching hospital in Brazil

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Abstract

The aim of this study was to evaluate the prevalence and antimicrobial resistance profile of Gram-positive cocci and Gram-negative bacilli isolated from the surgical environment. All samples were collected during the intraoperative period of clean/clean-contaminated (G1) and contaminated (G2) surgery. A total of 150 samples were collected from the surgical wound in the beginning (n = 30) and end (n = 30) of the procedure, surgeon's hands before (n = 30) and after (n = 30) antisepsis and the surgical environment (n = 30). Forty-three isolates with morphological and biochemical characteristics of *Staphylococcus* spp. and 13 of Gram-negative bacilli were obtained. Coagulase-negative staphylococci (85.71% [18/21]), coagulase-positive staphylococci (9.52% [2/21]) and *Pseudomonas* spp. (47.52% [1/21]) in G1, and coagulase-negative staphylococci (40% [14/35]), coagulase-positive staphylococci (20% [7/35]), *Proteus* spp. (17.14% [6/35]), *E. coli* (8.57% [3/35]), *Pseudomonas* spp. (2.86% [1/35]) and *Salmonella* spp. (2.86 [1/35]) in G2 were more frequently isolated, and a high incidence of multidrug resistance was observed in coagulase-negative staphylococci (87.5% [28/32]), coagulase-positive staphylococci (100% [11/11]) and Gram-negative bacilli (76.92% [10/13]). Methicillin-resistant *Staphylococcus* spp. accounted for 83.72% (36/43) of the *Staphylococcus* strains. Gram-negative bacilli cefotaxime-resistance constituted 81.82% (9/11) and imipenem resistance constituted 53.85% (7/13). The high rate of resistance of commensal bacteria found in our study is worrying. Coagulase-negative staphylococci are community pathogens related to nosocomial infections in human and veterinary hospitals, their presence in healthy patients and in veterinary professionals represent an important source of infection in the one health context. Continuous surveillance and application of antimicrobial stewardship programs are essential in the fight against this threat.

Introduction

The infection caused by multidrug-resistant bacteria (MDR) presents a main challenge in the one health context because it increases morbidity, mortality and the costs related to healthcare (Suthar et al. 2014; Schwarz et al. 2016; McEwen and Collignon, 2018). The zoonotic potential of most MDR bacterial species related to serious infection highlight veterinary medicine as a relevant axis in the fight against to dissemination of these pathogens (Guardabassi et al. 2004a; Guardabassi et al. 2004b; Brusselaers et al. 2011; Suthar et al. 2014; Madec et al. 2017; McEwen and Collignon 2018; Grönthal et al. 2018; Shoen et al. 2019).

Methicillin-resistant *Staphylococcus* (MRS), extended-spectrum β -lactamases (ESBL) Enterobacteriaceae species, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are commonly cause of surgical site infections in pets and humans. These bacterial groups are the most prevalent microorganism acquired during the hospitalisation in the intensive care unit in human and veterinary medicine (Gandolfi-Decristophoris et al. 2012; Turk et al. 2014; Andrade et al. 2016; Walther et al. 2016; Chaudhary et al. 2017; Kaspar et al. 2018; McEwen and Collignon 2018; Shoen et al. 2019).

One of the main risk factors to post-surgical infection in dogs are the bacterial colonisation of skin, mucous and gastrointestinal tract by MDR bacteria and the presence of these pathogens in the operating room (Chauveaux 2015; Andrade et al. 2016; Burgess 2019). Furthermore, the introduction of these strains in the hospital environment poses a big threat to health care professionals, which become carriers and disseminators of MDR bacteria (Paul et al. 2011; Rodrigues et al. 2017; Kaspar et al. 2018).

Therefore, the aim of this epidemiologic study was to evaluate the incidence and antimicrobial resistance profile of Gram-positive cocci and Gram-negative bacilli present in the superficial surgical site from dogs, in surgeon's hands and in the surgical environment during the intraoperative period.

Material And Methods

Case selection

Thirty dogs, without antimicrobial therapy in the last 72 hours, undergoing to clean/clean-contaminated (G1 [n=10]) and contaminated (G2 [n=20]) surgery (WHO 2016) in a veterinary teaching hospital of São Paulo state, Brazil, during the 2019 year, were included in this study. Eight surgeons without symptoms of infection were included in this study. The owners of dogs and surgeons selected for this study signed informed consent for their participation.

Patient and surgical team preparation

The inducing and maintaining anaesthesia protocol were performed individually by small animal anaesthesiology care according to the *American Society of Anaesthesiologists* classification (Mayhew 2019). Cefazolin sodium (25mg/kg [IV]) was administered between 30 and 60 minutes before surgical incision. Hand's antisepsis was performed with 2% chlorhexidine solution or 2% povidone iodine.

Hair removal by clipping was initiated in the surgical preparation room and finalized after induction in the operating room. Surgical site antisepsis was performed with 2% chlorhexidine solution, 70% isopropyl alcohol and 0,5% alcoholic chlorhexidine solution.

Sample collection

Specimens were collected using a dry sterile cotton-tipped swab and transported in a sterile tube with 0,1% peptone salt solution. Sixty samples were collected from the hands surface of the main surgeon immediately before (C1 [n=30]) and after (C2 [n=30]) antisepsis. Swabs were rubbed in a circular motion from the wrists to the fingertips (back and palm of the hand), this procedure was repeated three times toward each finger by the same people with sterile gloves in all collections.

Immediately after the skin incision (M1 [n=30]) and previously to cutaneous closure (M2 [n=30]), the swab was rubbed in the superficial surgical site (skin and subcutaneous tissue) from the patient. The specimens of the surgical site were double collected. A Petri plate with Brain Heart Infusion agar (BHI;

Oxoid®, United Kingdom) was positioned nearby and in the same height to the surgery table during the procedure.

Bacteriological culture

Immediately after the procedure, each sample was spread onto MacConkey agar (Oxoid®, United Kingdom) and 5% bovine blood agar (Oxoid®, United Kingdom) and, together with the BHI environment plate, were incubated at 37°C for 24h, under aerobic conditions. Three morphologically distinct colonies were collected from each plate. Each strain was classified by cellular morphology, Gram staining, presence or absence of spores, and biochemical techniques (Barrow and Feltham 1993).

Catalase test was performed to identify bacterial genera of *Staphylococcus* spp. and *Streptococcus* spp. Coagulase test was performed with lyophilized rabbit plasma (Coagu-plasma®, Laborclin, Brazil) for the *Staphylococcus* spp. strains. Gram-negative strains were spread in Triple Sugar Iron agar (TSI; Oxoid®, United Kingdom) and incubated at 35°C for 18-24h, under aerobic conditions. Urease test was performed for the *Salmonella* spp. and *Proteus* spp. strains and citrate tests were performed for *E. coli* and *Klebsiella* spp. strains (Koneman et al. 2001).

Antimicrobial susceptibility tests

The antimicrobial susceptibility test was performed and inhibition zone diameters were measured following CLSI standard guideline (CLSI 2013; CLSI 2015). The antimicrobials disks (DME®, Brazil) used in the test are described in Table 1. Clindamycin and erythromycin antimicrobial disks were placed at a distance of 15mm to perform inducible clindamycin resistance testing (D-test).

Table 1 Drugs used in the antimicrobial susceptibility test

Legend: *The italic classes were used to classified multidrug resistance.

The proportion of resistance was obtained by analysing the specific resistance of each identified pathogen to every antimicrobial tested (% R). The intermediate results were considered to be resistant to the data analyses since the outcome of treatment for these cases directly depended on the antimicrobial dose and the site of infection (CLSI 2015).

The phenotypic presence of the *mecA* gene (MRS) was assigned to the pathogens which exhibited oxacillin resistance (Bemis et al. 2009; Wu et al. 2016; Naccache et al. 2019). The phenotypic presence of the *ermA* gene (MLS_B phenotype) was assigned to *Staphylococcus* spp. strains which exhibited erythromycin resistance and a D-shape zone around the clindamycin in the area between the two disks (Fiebelkorn et al. 2003). MDR was confirmed when a strain was resistant to at least 1 drug belonging to 3 or more antimicrobial classes (Magiorakos et al. 2012; Basak et al. 2016).

Statistical analysis

A descriptive analysis of the infection sites, pathogens, and resistance proportion was performed. The chi-square test was used to compare bacterial resistance rate. All analyses were performed using R[®] software 3.3.0 (R Foundation for Statistical Computing, Austria). The significance threshold was set at 0.05.

Results

Bacterial contamination

Growth of Gram-positive cocci and Gram-negative bacilli in G1 was observed in 11/20 (55%) and 1/20 (5%) procedures, respectively, in at least one moment of collect. In the G2, 7/10 (70%) procedures presented Gram-positive cocci strains and 5/10 (50%) procedures presented Gram-negative bacilli strains, in at least one moment of collect. The number of strains obtained in G1 was less than G2 ($p=0.0122$). Statistical difference in the different moments of collect was observed only in the proportion of strains obtained from surgical sites in M1 ($p=0.007$). The bacteria growth in both groups was described in Table 2.

Table 2 Number and proportion of strains obtained from environment, surgeon's hands and superficial surgical site

Legend: C1=surgeon's hands before antisepsis, C2=surgeon's hand after antisepsis, M1=initial moment, M2=final moment. Significance level was set at $p<0.05$.

Fifty-six strains of Gram-positive cocci and Gram-negative bacilli were obtained from 150 samples, of which 21/56 (37.5%) were obtained in the G1 and 35/56 (62.5%) in the G2. Morphological and biochemical characteristics of *Staphylococcus* spp. were observed in 43/56 (76.79%) strains, of which 32/43 (74.42%) were coagulase-negative staphylococci (CoNS) and 11/43 (25.58%) were coagulase-positive staphylococci (CoPS). Gram-negative bacilli characteristics was observed in 13/56 (23.21%) strains, which of 6/56 (10.71%) was classified as *Proteus* spp., 3/56 (5.36%) as *E. coli*, 2/56 (3.57%) as *Pseudomonas* spp., 1/56 (1.88%) as *Klebsiella* spp. and 1/56 (1.88%) as *Salmonella* spp.

In the Gram-positive cocci strains, there was a higher number of CoNS in both groups, of which 18/20 (90%) were in G1 and 14/23 (60.87%) in G2. G2 had a higher number of CoPS strains (9/23 [39.13%]) than G1 (2/20 [10%]). In the G1, the most frequent strains were CoNS (18/21 [85.71%]), of which 9/18 (50%) were isolated from environment, 5/18 (27.78%) from surgeon's hands before antisepsis and 4/18 (22.22%) from surgical site in M2. In the G2, CoNS was also the most frequent strains, which of 4/14 (28.57%) were isolated from environment, 4/14 (28.57%) from surgical in M1, 4/14 (28,57%) from surgical site in M2 and 2/14 (14.29%) from surgeon's hands before antisepsis (Figure 1).

Fig. 1 Percentage distribution of Gram-positive cocci and Gram-negative bacilli obtained from the environment, surgeon's hands and superficial surgical site during the perioperative period. Clean and clean-contaminated surgery (A); Contaminated surgery (B)

Legend: ENV=environment, C1=surgeon's hands before antiseptics, C2=surgeon's hand after antiseptics, M1=initial moment, M2=final moment, CoNS=coagulase-negative staphylococci, CoPS-coagulase-positive staphylococci.

The only Gram-negative bacilli in G1 was obtained from the environment and it was classified as *Pseudomonas* spp. (1/21; 4,76%). Gram-negative bacilli strains in the G2 isolated from surgeon's hands were classified as *Proteus* spp. (3/12 [25%]), *Pseudomonas* spp. (1/12 [8.33%]) and *Salmonella* spp. (1/12 [8,33%]) and from surgical site were classified as *Proteus* spp. at M1 (2/12 [16.67%]), *E. coli* at M1 (2/12 [16.67%]) and M2 (1/9 [74.55%]) and *Klebsiella* spp. at M1 (1/12 [8,33%]) (Figure 1).

Antimicrobial resistance

Oxacillin (47/54 [87.04%]) and erythromycin (37/43 [86.05%]) resistance were the most frequent in our strains (Figure 2). MDR was observed in 49/56 (87.50%) strains.

Fig. 2 Percentage distribution of antimicrobial resistance in Gram-positive cocci and Gram-negative bacilli obtained from the environment, surgeon's hands and superficial surgical site during the perioperative period. Macrolides, lincosamides and fluorquinolones (A); Aminoglycosides, glycopeptides and tetracycline (B); Cephalosporins and carbapenems (C); Penicillins (D)

Legend: AZI = azithromycin, ERI = erythromycin, CLI = clindamycin, ENO = enrofloxacin, LEV = levofloxacin, NOR = norfloxacin, GEN = gentamicin, AMI = amikacin, TEC = teicoplanin, TET = tetracycline, SUT = trimethoprim-sulfamethoxazole, CLO = Chloramphenicol, CFE = cephalexin, CEF = cefazolin, CFO = cefoxitin, CRO = ceftriaxone, CAZ = ceftazidime, CTX = cefotaxime, IPM = imipenem, PEN = penicillin, AMO = amoxicillin, AMC = amoxicillin-clavulanate, AMP = ampicillin, APS = ampicillin-sulbactam, OXA = oxacillin, MDR = multidrug-resistance.

CoNS strains presented higher resistance proportion for erythromycin (29/32 [90.63%]) and CoPS strains for ampicillin (10/11 [90.91%]) and enrofloxacin (10/11 [90.91%]). *Staphylococcus* spp. oxacillin-resistance was observed in 36/43 (83.72%) strains, of which 7/11 (63.64%) were CoPS and 29/32 (90.63%) were CoNS. Erythromycin and clindamycin resistance were observed in 37/43 (86.05%) and 25/43 (58.14%) of staphylococci strains. Of the 37 erythromycin-resistance *Staphylococcus* spp. strains, 3 (8.11%) were positive to D-test. Thirty-nine of 43 (90.70%) *Staphylococcus* spp. strains were classified as MDR, of which 11/11 (100%) were CoPS and 28/32 (87.50%) were CoNS (Figure 2). There was no statistical difference found between the proportion of oxacillin-resistance and MDR of each group (p=0.9417 and p=1, respectively) and of each site (p=0,9805 and p=0,3448, respectively) in the *Staphylococcus* spp. strains (Table 1).

Gram-negative bacilli presented higher proportion of resistance for clindamycin (11/11 [100%]), oxacillin (11/11 [100%]), cefotaxime (9/11 [81.82%]) and tetracycline (9/11 [81.82%]). MDR was observed in 10/13 (76.92%) of the Gram-negative bacilli strains (Figure 2).

Discussion

Our study describes the resistance profile of Gram-positive cocci and Gram-negative bacilli isolates from superficial surgical sites, surgeon's hands and operating room. *Staphylococcus* spp. were the most frequent species in samples from clean/clean-contaminated (20/21 [95.24%]) and contaminated (22/35 [62.86%]) surgery. The most of Gram-negative bacilli were obtained from G2 (11/12 [91.67%]), of which 7/12 were of *Proteus* spp. (58.33%), 4/12 of *E. coli* (33.33%) and 3/12 of *Pseudomonas* spp. (25%). MDR were high in CoPS (11/11 [100%]) and Gram-negative bacilli (10/13 [76.92%]) presented high proportion of oxacillin and clindamycin-resistance (11/11 [100%]) (Fig. 2).

Contaminated surgeries showed a high number of Gram-positive cocci (70%) and Gram-negative bacilli (50%), which is expected due to the higher rate of bacterial contamination of the superficial surgical site in these procedures (Nelson 2011; Turk et al. 2014; Chaudhary et al. 2017).

Gobbo et al. (2017) and Menezes et al. (2020) reported the same pathogens and high proportion of antimicrobial resistance in samples of surgical environment and clinical infections in a veterinary teaching hospital in São Paulo, Brazil. *Staphylococcus* spp. was the most frequent species reported in other veterinary teaching hospitals in other states in Brazil (Fernando et al. 2015; Murta et al. 2015). Although the pathogens were similar in these studies, the difference in the bacterial prevalence, mainly between different states, underscores the local and temporal dynamics of the bacterial epidemiology.

The bacterial species observed in this study were the most prevalent related to the surgical site infections in dogs in Brazil and other countries (Suthar et al. 2014; Fernando et al. 2015; Murta et al. 2015; Verwilghen and Ameet 2015, Andrade et al. 2016). *Staphylococcus* spp. are commensal pathogens from skin and mucosa of humans and animals (Brusselaers et al. 2011; Paul et al. 2011; Paul et al. 2012), what justifies the high prevalence of these microorganisms in samples of the surgical site and surgeon's hands in both groups (Fig. 1).

We observed more CoNS strains (32/43 [74.42%]) than CoPS (11/43 [25.58%]), and CoNS was most prevalent than CoPS in G1 ($p < 0.05$). This fact may be justified due CoNS strains, mainly *S. epidermidis*, are opportunistic pathogens frequently found in dog's and human's skin and usually is not related to infectious in health patient (Kern and Perrenten 2013; Wu et al. 2016; Shoen et al. 2019). *S. pseudointermedius* and *S. aureus* are frequently reported in cutaneous infections in dogs, what is usually observed in superficial surgical site in the contaminated surgery (Guardabassi et al. 2004a; Howe and Boothe 2006; Hillier et al. 2014; Singh and Weese 2017).

MRS strains present resistance to all β -lactam drugs due to the presence of the *mecA* gene (Bemis et al. 2009; Wu et al. 2016; Naccache et al. 2019). In the present study, oxacillin-resistance was most prevalent in CoNS (90%) than CoPS (64%), which is concerning because these species have become potential pathogens related to sepsis in intensive care units (ICU). Furthermore, these strains are associated with biofilm formation and can cause post-surgical infections until one year after the procedure (Becker et al. 2014; Naccache et al. 2019). Becker et al. (2014) reported surveillance of 89% in methicillin-resistance

CoNS in humans' hospitals, which was also observed in our study (Fig. 2). The high proportion of oxacillin-resistance in our strains could lead to surgical antimicrobial prophylaxis failure, which is usually performed with first and second-generation of cephalosporins (Boothe and Boothe 2015; Singh and Weese 2017).

Three of 37 (8.11%) *Staphylococcus* spp. strains, that were resistant to erythromycin, presented inducible clindamycin resistance (D-test). This fact can occur due to the presence of *erm* gene, which encodes resistance to macrolides, lincosamides and streptogramin B, called the MLS_B phenotype (Fernandes et al. 2007). The proportion of strain positive to D-test in our study was more than other reports in North America (Faires et al. 2009; Gold and Lawhon 2013) and less than health workers in North Africa (Mahmoud et al. 2015).

Fluoroquinolone resistance (Fig. 2) was between 54–91%, highlighting Gram-negative bacilli resistance to enrofloxacin (91%). This drug is usually used to treat serious infections caused by enterobacteria strains and *Pseudomonas* spp. in veterinary medicine (Papich 2013; Feng et al. 2019; Pang et al. 2019). Your empiric administration could contribute to the resistance to other antimicrobials of the same class used to treat human infections, as ciprofloxacin (Barrasa et al. 2000; Papich 2013). In this study, we observed similar proportions of resistance between the drugs of this class (Fig. 2).

The gastrointestinal tract of dogs and humans are colonized by bacteria from the Enterobacteriaceae family. The presence of these pathogens in the hospital environment and skin may indicate a failure in cleaning and disinfection of these surfaces (Cinquelpalm et al. 2013; Suthar et al. 2014; Drzewiecka 2016). In this study, we observed that in G2 the strains of these pathogens were larger (13/35 [37.14%]) than in G1, in which no strain was obtained. The most frequent species were *Proteus* spp. (6/10 [60%]) and *E. coli* (3/10 [30%]).

The presence of these strains, mainly *Proteus* spp., in the surgical site and surgeon's hands is probably due to cross-infection and could suggest a failure in antisepsis techniques (Drzewiecka 2016). Chlorhexidine compounds are more effective against Gram-positive than Gram-negative strains, which may justify the persistence of enterobacteria after biocide application (Karpinski and Szkaradkiewicz 2015; Belo et al. 2018).

ESBL production is the mainly mechanism of resistance in Enterobacteriaceae family, which results in penicillin, cephalosporin, and aztreonam hydrolysis. β -lactamase inhibitors, such as clavulanic acid, sulbactam, and tazobactam could inactivate your action, and these strains are usually sensitive to carbapenem class. The cefotaxime-resistance could be used to detect the phenotypic production of this enzyme (CLSI 2015; Laudy et al. 2017). Gram-negative bacilli obtained in our study presented high prevalence (82%) of cefotaxime-resistance.

Carbapenemase-production is another important resistance mechanism of Enterobacteriaceae. These strains presented resistance to all drug of the β -lactam class (Seibert et al. 2014). The high prevalence of imipenem-resistance (54%), a carbapenem class-drug, in our study is worrying because this drug is used

to treat serious infections in human medicine. Furthermore, the proportion of MDR strains were high in our strains of CoNS (88%), CoPS (100%) and Gram-negative bacilli (77%), which could mean high probability in treatment failure (Madec et al. 2017).

The presence of MDR strains in healthy patients and in the environment is frequently reported in the literature (Chah et al. 2013; Priyantha et al. 2016, Dupouy et al. 2019; Ortega-Paredes et al. 2019), as well as the possibility of transmission to the veterinarians (Paul et al. 2011; Rodrigues et al. 2017). Thus, the patient, health professional and environment's colonisation for these strains, highlighting MRS, ESBL Enterobacteriaceae and carbapenemase-producing Enterobacteriaceae, could represent a high risk to human and veterinary patient due therapy failure, and to public health because they are important silent sources of infection (Guardabassi et al. 2004a; Guardabassi et al. 2004b, Becker et al. 2014; Suthar et al. 2014). Furthermore, these strains need, urgently, development for new drugs to treat efficiently these infections (McEwen and Collignon 2018).

This study has some limitations. Although morphological and biochemical tests are not the most sensitive alternative for diagnosing species and bacterial strains, they are financially viable and technically simpler than molecular tests, as a polymerase chain reaction, 16S rRNA gene sequencing, and pulsed-field gel electrophoresis (Ribot et al. 2006; Woo et al. 2008; Srinivasan et al. 2015). The results obtained in the bacteriological culture and antimicrobial susceptibility tests are important in clinical care and they are essential in an epidemiological database for the development of antimicrobial stewardship program and in epidemiological surveillance in the one health context.

Conclusion

This study identified a high rate of methicillin-resistant CoNS (90%), Gram-negative bacilli resistant to cefotaxime (82%) and MDR strains (87.50%). Furthermore, we observed a high resistance proportion in drugs used to treat serious infections in humans, as teicoplanin (37.21%) and imipenem (54%). These high rates of resistance are worrying because these strains were collected in skin surfaces without infectious, and the pathogens described here are frequently reported in human and veterinary surgical site infection and intensive care units. Beyond the high potential for therapy failure due to infections for MDR bacteria, we observed the veterinary surgical environment as potential reservoir and disseminator of these pathogens, mainly of community species, as MRS.

Finally, this study highlights the need of control and prevention in MDR strains transmission in healthcare, that address the topic according to the One Health concept and included veterinary medicine as an important cornerstone in the fight against this threat.

Declarations

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Consent to participate

All authors participated voluntarily in the research

Consent to publish

All authors drafted, revised, and approved the submitted manuscript.

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Availability of data and materials

All data and material are available for publication.

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Tables

Table 1 Drugs used in the antimicrobial susceptibility test

Class*	Sub-class	Agent
<i>Aminoglycosides</i>		Amikacin (30µg), Gentamicin (10µg)
<i>β-lactam</i>	First-generation cephalosporin	Cephalexin (30µg), Cefazolin (30µg)
	Second-generation cephalosporin	Cefoxitin (30µg)
	Third-generation cephalosporin	Cefotaxime (30µg), Ceftriaxone (30µg), Ceftazidime (30µg)
	<u>Carbapenem</u>	Imipenem (10µg)
	Penicillin	Amoxicillin (10µg), Amoxicillin-clavulanate (10µg), Ampicillin (10µg), Ampicillin-sulbactam (20µg), G Penicillin (10 µg), Oxacillin (1µg)
<i>Amphenicol</i>		Chloramphenicol (30µg)
<i>Lincosamide</i>		Clindamycin (5µg)
<i>Fluorquinolones</i>	Second-generation	Ciprofloxacin (5µg), Enrofloxacin (5µg), Norfloxacin (5µg)
	Third-generation	Levofloxacin (5µg)
<i>Sulphonamides</i>		Sulfamethoxazole and trimethoprim (25µg)
<i>Tetracycline</i>		Tetracycline (30µg)
<i>Glycopeptide</i>		Teicoplanin (30µg)
<i>Macrolides</i>		Azithromycin (15µg), Erythromycin (15µg)

Legend: *The italic classes were used to classified multidrug resistance.

Table 2 Number and proportion of strains obtained from environment, surgeon's hands and superficial surgical site

	G1 (n=21)		G2 (n=35)		<i>p-value</i>
	N	%	N	%	
Environment	10	47,62	7	20	0,0606
C1	7	33,33	5	14,29	0,1785
C2	0	0	2	5,71	0,71
M1	0	0	12	34,29	0,007
M2	4	19,05	9	25,71	0,8063
TOTAL	21	38,89	35	64,81	0,0122

Legend: C1=surgeon's hands before antisepsis, C2=surgeon's hand after antisepsis, M1=initial moment, M2=final moment. Significance level was set at $p < 0.05$.

Figures

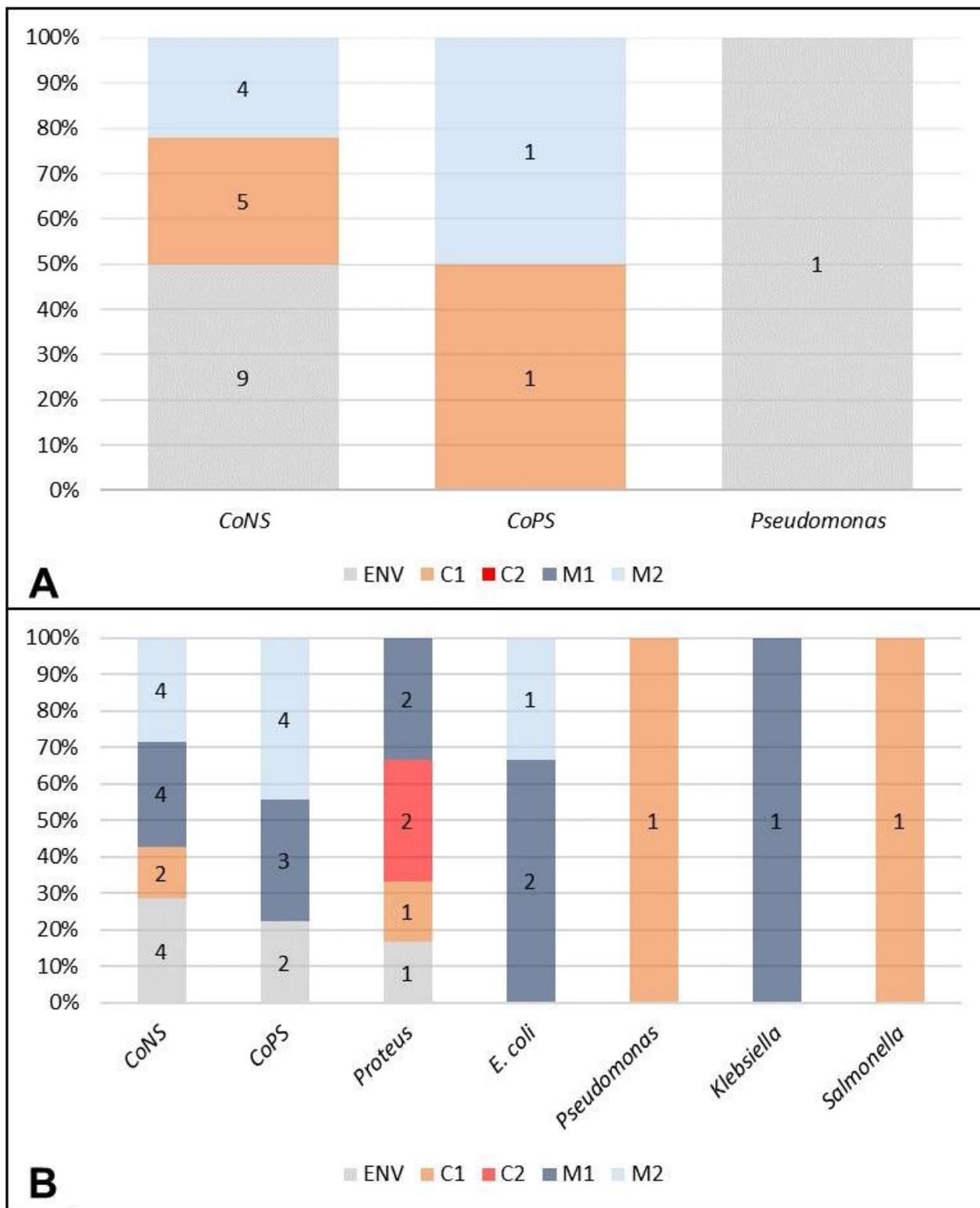


Figure 1

Percentage distribution of Gram-positive cocci and Gram-negative bacilli obtained from the environment, surgeon's hands and superficial surgical site during the perioperative period. Clean and clean-contaminated surgery (A); Contaminated surgery (B) Legend: ENV=environment, C1=surgeon's hands before antisepsis, C2=surgeon's hand after antisepsis, M1=initial moment, M2=final moment, CoNS=coagulase-negative staphylococci, CoPS-coagulase-positive staphylococci.

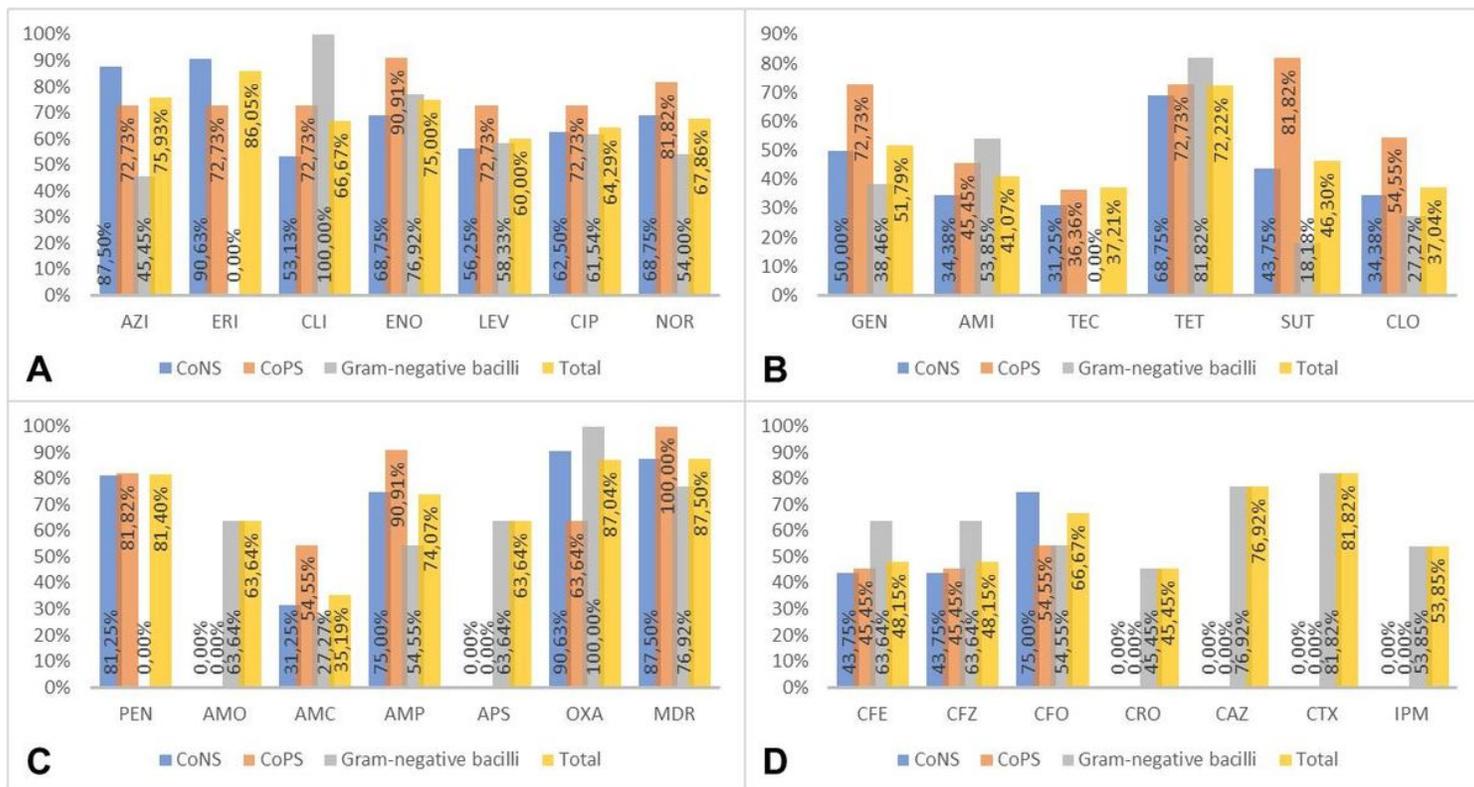


Figure 2

Percentage distribution of antimicrobial resistance in Gram-positive cocci and Gram-negative bacilli obtained from the environment, surgeon's hands and superficial surgical site during the perioperative period. Macrolides, lincosamides and fluorquinolones (A); Aminoglycosides, glycopeptides and tetracycline (B); Cephalosporins and carbapenems (C); Penicillins (D) Legend: AZI = azithromycin, ERI = erythromycin, CLI = clindamycin, ENO = enrofloxacin, LEV = levofloxacin, NOR = norfloxacin, GEN = gentamicin, AMI = amikacin, TEC = teicoplanin, TET = tetracyclin, SUT = trimethoprim-sulfamethoxazole, CLO = Chloramphenicol, CFE = cephalexin, CEF = cefazolin, CFO = ceftiofuran, CRO = ceftriaxone, CAZ = ceftazidime, CTX = cefotaxime, IPM = imipenem, PEN = penicillin, AMO = amoxicillin, AMC = amoxicillin-clavulanate, AMP = ampicillin, APS = ampicillin-sulbactam, OXA = oxacillin, MDR = multidrug-resistance.