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Original article

Resistance profile of bacteria isolated from the environment of high-risk departments in Ziguinchor hospitals

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ABSTRACT

Background: The environment of our hospital facilities is colonised by various microorganisms. These microorganisms in general and bacteria in particular are often responsible for nosocomial infections. The occurrence of these infections is linked firstly to the lack of asepsis, secondly to the nature of the colonising bacteria and thirdly to the immune status of the patients. The objective of this study was to evaluate the composition of the bacterial flora and to determine the antibiotic resistance profile of these bacteria. **Methods :** We proceeded to swab the surface of the work areas (table, bench, trolley, hospital bed, door wrist, gurney, incubator, respirator, etc.). The swabs were then soaked in a culture broth (Thioglycolate Broth or BT) for 18 to 24 hours before being plated on selective media for identification ; Chapman agar, EMB agar (Eosin Methylen Blue), GSN agar (Blood Agar + nalidixic acid), Sabouraud agar. **Results:** The isolated bacteria consisted mainly of multidrug-resistant bacteria (MDR). Thus, extended-spectrum beta-lactamase-secreting bacteria represented 5.5% (8 strains) of the isolated bacteria were distributed as follows: *Enterobacter spp* (25%, n=4) *Klebsiella pneumoniae* (12.5%, n=2) and *Escherichia coli* (12.5%, n=2). Among the other BMR, we found *Acinetobacter spp* (25%, n=4), *Pseudomonas aeruginosa* (6.25%, n=1) and methicillin-resistant *Staphylococcus aureus* (18.75%, n=3). **Conclusion:** Nosocomial infections are nowadays one of the main causes of prolonged hospital stay. The isolated bacteria of medical interest were mainly multidrug resistant bacteria. It is therefore imperative to respect the rules of hygiene during care and to evaluate the composition of the bacterial flora of the services in order to set up a strategic plan to fight against these infections.

Introduction

The environment of our hospitals is constantly colonised by microorganisms of varying degrees of pathogenicity. These microorganisms in general and bacteria in particular are often responsible for nosocomial infections. The service in question, the immune status of the patients, the virulence and antibiotic resistance of the bacteria are determining factors in the occurrence of nosocomial

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infections. The occurrence of nosocomial infections is linked first of all to the lack of hygiene during care procedures, then to the nature of the colonising bacteria and finally to the condition of the patients. These nosocomial infections are a real concern because of their frequency, their severity and the antibiotic resistance of the bacteria involved, not to mention the medico-legal aspect that they raise [1], as well as the additional economic cost due to the increase in the length of hospitalisation.

In the semi-urban environment, this is still a subject that has never been documented and is therefore poorly controlled, although these infections exist and are frequent in hospital wards.

We therefore took samples from the environment of high-risk departments to assess the composition of the bacterial flora and to determine the antibiotic resistance profile of bacteria found in two hospitals in the Ziguinchor region (southern Senegal).

Method

We took 146 samples from 15 April to 15 May 2021 from very high-risk departments and departments at risk of nosocomial infections in two hospitals in the Ziguinchor region: the Hôpital de la Paix and the Hôpital régional.

The very high-risk departments at the Ziguinchor Peace Hospital were: intensive care and neonatology.

The high-risk departments at the Ziguinchor Peace Hospital were: operating theatre, nephrology, gynaecology, surgery and dermatology.

The very high-risk departments at the Ziguinchor Regional Hospital were: intensive care, neonatology, orthopaedics and neurosurgery.

The high-risk departments at the Ziguinchor Regional Hospital were: nephrology (haemodialysis), operating theatre, gynaecology and surgery.

We swabbed the surface of the work areas (table, bench, trolley, hospital bed, door wrist, gurney, incubator, respirator, etc.)

Sampling was carried out between 9am and 10am; which corresponds to the hours of the visit. The sampling points were those closest to the patient. Swabbing is a non-standardised technique, less sensitive than the use of the contact box, but rapid and easily applicable to irregular surfaces such as those selected in our study [2].

The method consists of moistening a sterile swab in physiological water and rubbing a delimited area of 25 cm^2. The swabs are then soaked in a tube containing thioglycolate broth for 18 to 24 hours before being subcultured onto the selective media [2]. :

- Eosin Methylen Blue Agar (EMB): Isolation of fermentative and non-fermentative Gram-negative Bacilli
- Chapman's agar: Gram positive cocci (*Staphylococcus* and *micrococcus*)
- Blood agar + nalidixic acid : Gram positive cocci (*Streptococcus*)

The inoculated media are incubated at 37°C for 18-24 hours and then observed for bacterial growth.

Identification of Gram-negative bacilli is done using the classical gallery composed of Kligler Hajna (KH), Manitol-mobility (MM), Simmons Citrate (SC), Urea-tryptophan and simple peptone water (PSE). For non-fermentative BGNs, the oxidase test and mobility were used to determine the species such as *Pseudomonas aeruginosa* and *Acinetobacter spp*.

The identification of Gram-positive cocci was done first by enzymatic orientation test with hydrogen peroxide (catalase test) and then by pathogenicity tests (free coagulase and DNAse test, 5µg Novobiocin sensitivity) and latex test (agglutination) according to family and genus.

The identification of bacillus and micrococcus was done solely on the basis of microscopy with long Gram-positive bacilli (bacillus) and large Gram-positive tetrad cocci (micrococcus).

Antibiotic susceptibility testing was performed by the Mueller-Hinton (MH) agar diffusion method according to the standards and recommendations of the Antibiogram Committee of the French Microbiology Society (ACFMS2020).

The following discs (Bio-Rad, France) were tested for staphylococci: penicillin G (PENG-1 IU 6 µg (10 IU)), ampicillin (AMP- 2 µg) cefoxitin (FOX-30 µg), chloramphenicol (CHL-30 µg), tetracycline (TET-30 µg), kanamycin (KMN-30 µg), Tobramycin (TOB-10 µg), gentamicin (GMN-30 µg), erythromycin (ERY-15 µg), lincomycin (LCN-15 µg), pristinamycin (PTN-15 µg), pefloxacin (PEF-5 µg), norfloxacin (NOR-10 µg), ciprofloxacin (CIP-5 µg), fusidic acid (FAD-10 µg),

vancomycin (VAN-30 µg). *Escherichia coli* ATCC 25922 and *S. aureus* ATCC 29213 were used as reference strains for antibiotic disc control.

Penicillin G and cefoxitin resistance was used to determine the profile of methicillin-resistant (MRSA) or penicillinase-secreting *Staphylococcus.*

The following discs were tested for fermentative and non-fermentative Gram-negative bacilli **(Table 1).**

Testing for extended spectrum beta-lactamase (ESBL) was performed using a disc of Amoxicillin + clavulanic acid placed in the centre of the box surrounded by discs of 3rd generation cephalosporin (ceftriaxone, ceftatazidime, cefotaxime) at a distance of 20 mm from the central disc (AMC).

The disc matching technique makes it possible to demonstrate synergy between a beta-lactamase inhibitor (clavulanic acid) and a 3rd generation cephalosporin.

The determination of the species of enterobacteria is followed by the performance of an antibiogram. This is done by preparing a bacterial inoculum with a density equivalent to 0.5 McFarland. Then 1 ml of this suspension is added to 9 ml of sterile physiological water to obtain a 1/10 dilution. A Muller Hinton (MH) agar plate is plated with a swab from the 1:10 dilution.

The antibiotics discs were then placed directly on the cultures and the plates were incubated at 37°C. The reading was taken after 24 hours. Antimicrobial activities were assessed by measuring the inhibition diameters around the discs and interpretation was made using the critical values defined by the Antibiogram Committee of the French Society of Microbiology.

Table 1. List of antibiotics tested in the *Enterobacteriaceae* susceptibility test (ACFMS2020).

Antibiotics	**Abbreviations**	**Load (µg)**	**Critical diameters (mm)**	
			Φ ≥ D Sensitive	**Φ < d Resistant**
Amoxicillin	AMX	20	**19**	**19**
Amox + Ac. Clav	AMC	20 - 10	**19**	**19**
Ticarcillin	TIC	75	**23**	**23**
Piperacillin	PIP	30	**20**	**17**
Cefalotin	CF	30	**18**	**12**
Cefoxitin	FOX	30	**19**	**15**
Cefotaxime	CTX	5	**20**	**17**
Ceftriaxone	CRO	30	**23**	**20**
Ceftazidime	CAZ	10	**22**	**19**
Cefepime	FEP	30	**24**	**21**
Aztreonam	ATM	30	**24**	**21**
Imipenem	IPM	10	**22**	**16**
Chloramphenicol	C	30	**17**	**17**
Tobramycin	TOB	10	**17**	**14**
Gentamycin	GM	10	**17**	**14**
Amikacin	AN/ AK	30	**16**	**13**
Netilmicin	NET	10	**15**	**12**
Nalidixic acid	NA	30	**19**	**14**
Fosfomycin	FOS	200	**16**	**13**
Pefloxacin	PEF	5	**24**	**24**
Norfloxacin	NOR	10	**22**	**19**
Ciprofloxacin	CIP	5	**22**	**19**
Levofloxacin	LEV	5	**22**	**19**
Cotrimoxazol	Sxt/Cot	1,25/ 23,75	**16**	**13**
Colistin	CS	50	**15**	**15**

Results

- **Distribution of bacteria of medical interest isolated from hospital and department**

Bacteria usually isolated in human pathologies and frequently responsible for infections in patients received in hospital were considered to be of medical interest. We isolated them mainly from intensive care, gynaecology, neurosurgery and orthopaedics departments **(Table 2).**

- **Prevalence of isolated microorganisms**

Among the bacteria of medical interest, we isolated: *Staphylococcus spp* (11.6%, n=17), Enterobacteriaceae (5.5%, n=8) and Gram-negative Non-Fermentative Bacilli (3.4%, n=5) **(Figure 1).**

- **Distribution of strains according to bacterial species**

Of the 16 strains of medical interest found, Enterobacteriaceae represented 5.5% (8 strains) of the bacteria distributed as follows: *Enterobacter spp* (25%, n=4) *Klebsiella pneumoniae* (12.5%, n=2) and *Escherichia coli* (12.5%, n=2). Among the Gram-negative non-fermentative bacilli (5 strains), *Acinetobacter spp* accounted for 25% (n=4) *and Pseudomonas aeruginosa* 6.25% (n=1). For Gram-positive cocci, we found three (3) strains *of Staphylococcus aureus*, i.e. 18.75% **(Figure 2).**

- **Resistance phenotype of *Enterobacteriaceae* to beta-lactam**

Regarding the resistance phenotypes of Enterobacteriaceae, all *Enterobacter spp* isolates were of the wild type (inducible cephalosporinase), while all *Escherichia coli* and *Klebsiella pneumoniae* strains were secretors of an extended-spectrum betalactamase **(Figure 3)**.

- **Susceptibility profile of *Enterobacteriaceae to* antibiotics**

Resistance of *Enterobacteriaceae* to other antibiotics was most observed with fosfomycin, nalidixic acid (62.5%) and cotrimoxazole (50%) (**Figure 4).** Antibiotics from other families remained active on the *Enterobacteriaceae* strains found.

Table 2. Distribution of bacteria of medical interest isolated.

Hospitals	Services	Number of samples	Isolated bacteria of medical interest
HOSPITAL LA PAIX DE ZIGUINCHOR	Resuscitation	09	01
	Neonatology	11	00
	Operating theatre	11	00
	Nephrology	08	01
	Gynaecology	16	02
	Surgery	11	01
	Dermatology	00	00
ZIGUINCHOR REGIONAL HOSPITAL	Resuscitation,	10	04
	Neonatology	10	00
	Orthopaedics	09	03
	Neurosurgery	08	02
	Nephrology (haemodialysis)	07	00
	Operating theatre	15	00
	Gynaecology	09	02
	Surgery	12	00
TOTAL		146	16

Figure 1. Prevalence of isolated microorganisms.

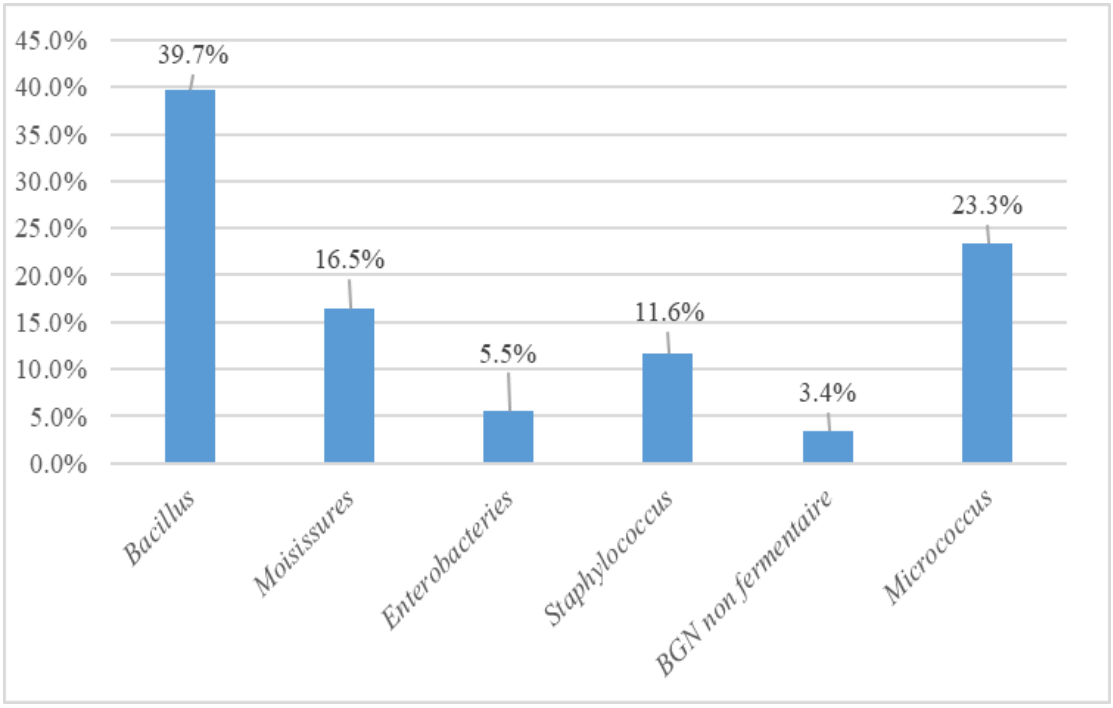


Figure 2: Distribution of strains by bacterial species

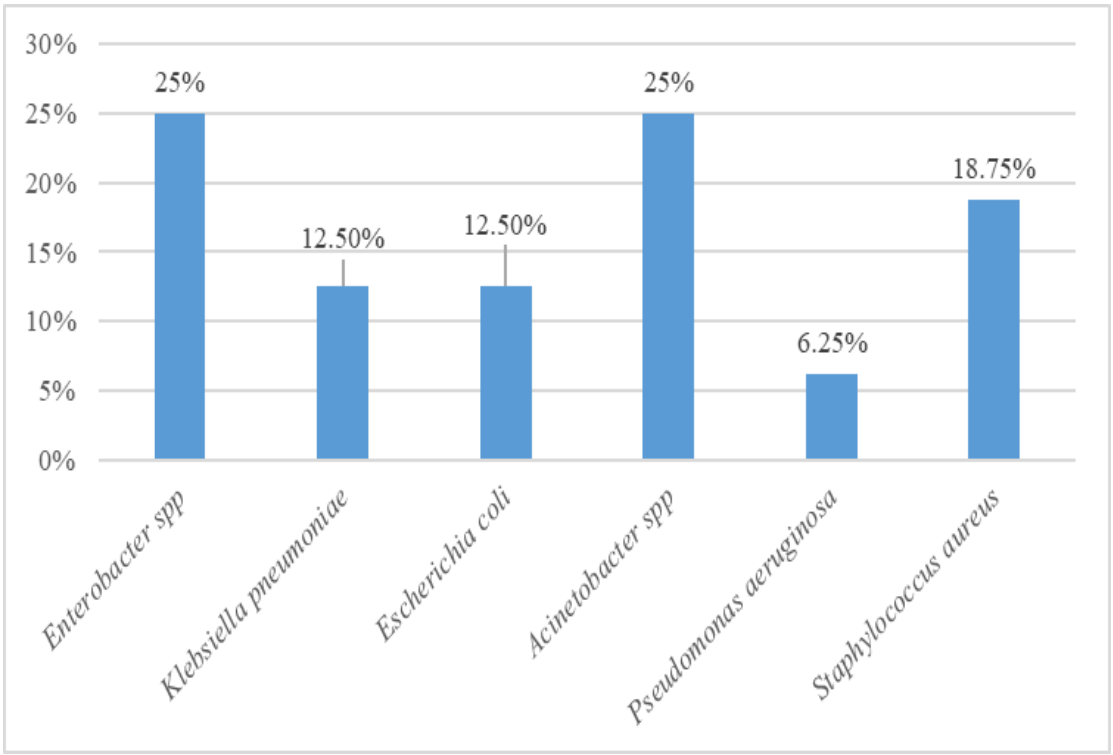


Figure 3. Resistance phenotype of *Enterobacteriaceae* to beta-lactam.

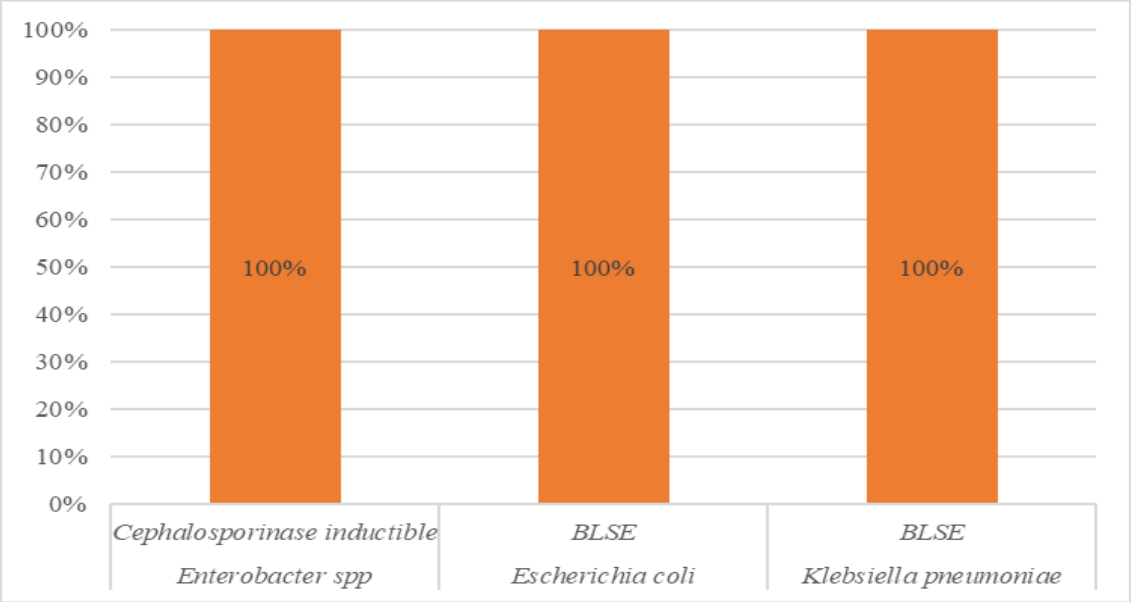
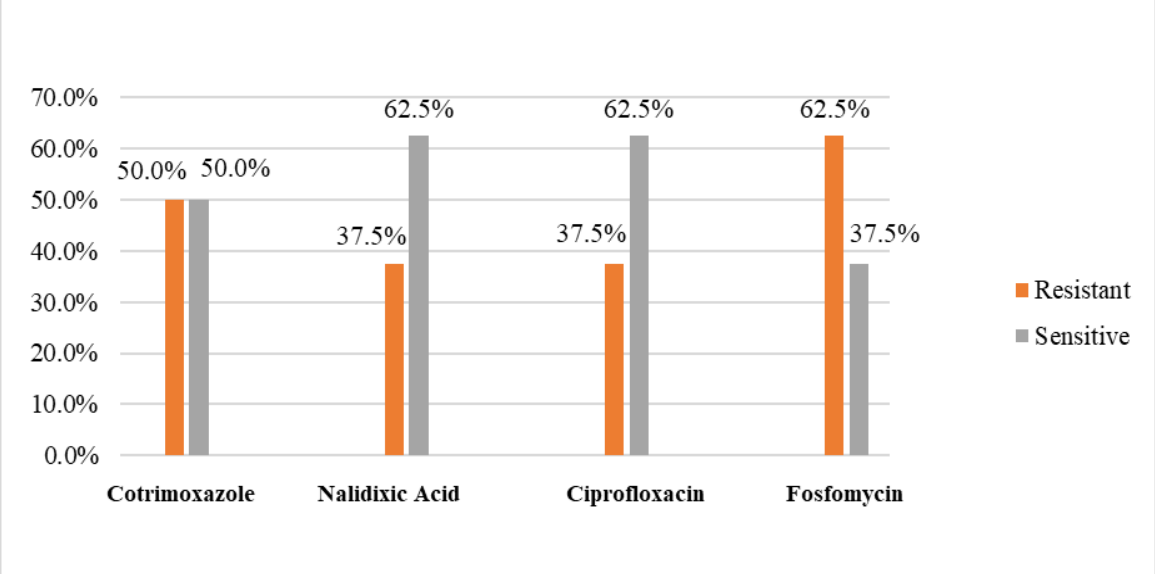


Figure 4. Susceptibility profile of *Enterobacteriaceae* to antibiotics.



Discussion

The colonisation of surfaces or care equipment by microorganisms is a source of nosocomial infection (NI). These infections are a real problem in our hospitals due to the working conditions.

The aim of our work was to evaluate the composition of the bacterial flora and to determine the antibiotic resistance profile of the isolated bacteria. The occurrence of infection depends on several parameters, including the diversity of the flora and the behaviour of bacteria to antibiotics. The "one day survey" is a rapid, inexpensive technique recommended by the WHO that allows the identification of the services most affected by this phenomenon in a given facility [3].

We consider bacteria of medical interest; bacteria usually isolated in human pathologies and frequently responsible for infections in hospital patients. In our study the bacteria of medical interest found were enterobacteria, non-fermentative Gram-negative bacilli and Staphylococcus. According to their resistance profile, we found Extended-Spectrum Bêta-Lactamase-Secreting Enterobacteria, methicillin-resistant *Staphylococcus*

aureus (MRSA), *Pseudomonas aeruginosa* and *Acinetobacter spp.*

Of all the enterobacteria (8 strains), we identified *Enterobacter spp* (4 strains), *Klebsiella pneumoniae* (2 strains) and *Escherichia coli* (2 strains). These enterobacteria found in the healthcare environment are second only to staphylococci among the bacteria in the environment of medical interest, and therefore may be the cause of healthcare-associated infections. Compared to a study carried out in a hospital in Ouagadougou [4] where Gram-negative bacilli were often the cause (67.4%) of healthcare-associated urinary tract infections with *Escherichia coli* and *Klebsiella spp* in 35.5% and 22% of cases respectively. In a study assessing the bacterial and fungal communities in the air in selected areas of the University Teaching Hospital, Kandy, Sri Lanka, it was found that *Escherichia coli* was isolated from the outpatient department, the operating theatre and respiratory disease unit and the bronchoscopy unit [5]. Other studies have concluded that *Escherichia coli* is the most common species found in the hospital environment [6,7].

Apart from *Enterobacteriaceae*, Staphylococci, especially the species *Staphylococcus aureus* has been found. In the previous study in Sri Lanka *Staphylococcus saprophyticus* represents one of the major nosocomial pathogens due to their typical opportunistic nature [5]. *Staphylococcus aureus* was mainly isolated from the intensive care unit of both hospitals. These strains can cause catheter-related bacteremia, septic shock, brain abscess, peritonitis, endocarditis, pneumonia, urinary tract infection, septic arthritis, and most commonly wound infection [8,9].

Bacterial ecology varies from one hospital, department or work area to another. Indeed, Yu et al. in China in their study on the characteristics of airborne microorganisms in the intensive care unit of the neurology department showed that the most common microorganisms were Gram-positive cocci [10]. The proportion of Gram-negative bacilli found in our study is lower than the proportion of gram-positive cocci found in the ward environment.

In addition to this problem of colonisation of the work surfaces of the departments by bacteria, there is also the problem of antibiotic resistance. Thus, among the bacteria isolated, we find multi-resistant bacteria such as: Extended-Spectrum Bêta-Lactamase-Secreting Enterobacteria, methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and *Acinetobacter spp*. These multi-resistant bacteria were mainly found in wards such as intensive care and maternity wards, which are "high antibiotic consumers". This resistance is the consequence of the selection pressure due to the use of antibiotics in these departments. Indeed, many hospitalised patients are under antibiotics for prophylactic or curative purposes. Thus, by preventing or treating a given infection with antibiotics, especially broad spectrum antibiotics, a population of antibiotic resistant bacteria is selected. These bacteria will eventually colonise patients, work surfaces, wards, and so on...

Indeed, avoiding or reducing the irrational use of antibiotics is a global objective that must be achieved through the improvement of practices by all health actors. In hospitals, strategies to prevent the spread of antibiotics are based on isolation and hygiene. These strategies are effective against the spread of certain Gram-negative bacteria, such as extended-spectrum beta-lactamase-producing Enterobacteriaceae, and have been much less effective against multi-resistant *Staphylococcus aureus,* which remains highly prevalent in French hospitals, whereas in Denmark the epidemic has been completely reduced [11]. The use of hydro-alcoholic solutions for hand disinfection could provide significant progress [12]. However, our best chance of controlling the evolution of bacterial resistance is to reduce antibiotic consumption [13,14].

Conclusion

This study is a first integrated approach for the evaluation of bacterial contamination in the risk departments of two hospitals in a semi-urban area, allowing simultaneously to have the bacterial ecology of the departments and the antibiotic resistance profile of these bacteria. It is important to respect asepsis during care but also to reduce as much as possible the irrational use of antibiotics in order to reduce the occurrence of nosocomial infections and the development of antibiotic resistance in bacteria.

Conflicts of interest: None.

Financial disclosure: None

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