INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 21, No. 6, p. 170-181, 2022

RESEARCH PAPER OPEN ACCESS

Antibiotic resistance pattern of pathogens causing nosocomial infections isolated from hospital environments in Algeria.

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Key words: Nosocomial infection, Antibiotic resistance, *Pseudomonas aeruginosa, Staphylococcus aureus, Enterobacteriaceae*

http://dx.doi.org/10.12692/ijb/21.6.170-181 Article published on December 05, 2022

Abstract

The emergence of resistance to antimicrobial agents is a global public health problem, particularly, pathogens causing nosocomial infections. This study aims to test the antibiotic resistance of strains isolated from hospital surfaces from six services at the Medea hospital in Algeria. In this context, we carried out a study on samples from the hospital environment. We collected 30 samples of which 32 strains were isolated and identified. Our results show the predominance of Gram-negative bacteria in nosocomial infections, but *S. aureus* was the most frequently isolated positive bacteria with a rate of 19%. The results show that 70% of the samples from the hospital environment were positive, of which beds and toilets had the highest rate. According to our study, the highest rate of resistant strains was observed in the male surgery (MS) and women's medicine (WM) services. The antibiotic resistance study selected 16 resistant strains. 11 strains are *Enterobacteriaceae*, 4 strains are *Staphylococcus aureus*, and one strain is *Pseudomonas aeruginosa*. The emergence of those bacteria constitutes a potential risk; however, the monitoring of the multi bacterial resistance has become a necessity and requires the establishment of an adapted strategy of intervention in order to avoid the risk of nosocomial infections.

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Introduction

Hospital-acquired infections are responsible for significant morbidity and mortality in healthcare environment. (National Audit Office, 2004). Globally, nosocomial infections are now a major public health problem, particularly in developing countries. They are serious complications of hospitalization. According to foreign, predominantly American statistics, 5-7% of hospitalized patients develop nosocomial infections (Berthelot *et al.,* 2005). However, the incidence of infections varies widely among NICUs. It occurs at an incidence of around 30% (Pessoa-Silva *et al.,* 2004) (Kawagoe *et al.,* 2001), and in developing countries, it is estimated to cause 40% of all neonatal deaths, (Zaidi *et al.,* 2005), depending on environmental factors and differences in clinical practice. (Borguesi and Stronati, 2008). During the last decade, there has been an alarming rise in hospital-acquired infections by multi-drug-resistant microorganisms (Livermore, 2000; Reacher *et al.,* 2000).

The discovery of antibiotics radically changed perceptions and ways of responding to infections: To a microbe, an antibiotic matched, and the equation was simple. However, probably as a result of over-prescribing and self-medication, as early as the 1950s and 1960s, many publications noted an increasing proportion of antibiotic-resistant germs this semi failure will surely be the driving force behind the organizational construction of the fight against nosocomial infection (Ellenberg, 2005). In hospitals, surfaces are regularly colonized by microorganisms, these microorganisms come from a variety of origins and may come from patients, health care providers, or visitors. These surfaces would thus constitute an ecological niche of multiresistant bacteria that can be a reservoir from which nosocomial infections can develop (Faye-Ketté, 2010). Nevertheless, nosocomial infections remain a major cause of preventable morbidity and mortality in developing countries where infection rates are relatively higher due to overcrowding of hospitals, poor infection control practices, lack of supervision, and inappropriate use of limited resources. (Emori and Gaynes, 1993). The major pathogens of neonatal infections differ not only from country to country and from nursery to nursery but also change within years in the same nursery. (Adams-Chapman and Stoll, 2007).

For these reasons, effective surveillance was very important to evaluate the epidemiology, associated risk factors, causative organisms, and outcomes based on understanding the epidemiology of nosocomial infection in our locality. Therefore, this study was conducted to determine the occurrence of nosocomial infections, associated risk factors, common microorganisms, to determine their antibiotic resistance patterns and the determination of their distribution according to the sampling sites in Mohamed Boudiaf Hospital in Medea, Algeria.

Sample collection

During the research, a total of 30 samples were collected from 6 services (Oncology, women's medicine, medicine man, gynecology, male surgery, and female surgery) at Mohamed Boudiaf hospital of Medea.

Isolation of nosocomial strains

The samples were collected from different surfaces (soils, beds, door handles, trolley, and sanitary) of the hospital environment by using the swab method. The samples were cultured and characterized using morphological and biochemical tests according to microbiological guidelines. Briefly, all samples were cultured on different agar mediums (Nutrient Agar, Mac Conkey, Cetrimide agar, Chapman Agar, hektoen agar, and Salmonella Shigella Agar). Finally, the cultured samples were incubated at 37 °C for 24–48 h.

Morphological diagnostic

Biochemical tests were performed to identify the agents isolated from the infections depending on the isolate type, sampling site, and Gram-positive or negative bacteria, catalase, and oxidase. Also, a coagulase test was used to identify *Staphylococcus aureus* by using rabbit plasma. (Andre *et al.,* 2008). Moreover, the indole diagnostic, Triple Sugar Iron

Agar (TSI), citrate, urea indole, and motility tests were used for the identification of the isolated bacteria. All chemicals, media, media components and other reagents were purchased from Sigma-Aldrich (USA).

Antibiotics susceptibility

The antibiotic susceptibility of the isolates was determined using Agar Disk Diffusion Method (MDDM) by Mueller-Hinton agar medium according to the Clinical & Laboratory Standards Institute (CLSI) instructions. For this purpose, a suspension of pure bacteria with a concentration of 0.5 McFarland (1.5 × 108 CFU.mL−1) was prepared in sterile saline and the antibiotic resistance of the isolates was evaluated against different antibiotic groups.

After the deposit of the antibiotic disks on the surface of the agar, the dishes were incubated for a minimum of 16 h at 37°C. The sensitivity to the antibiotics is thus dependent on the diameter of inhibition observed on the box. A preestablished concordance curve is used to determine the MIC based on the measured inhibition diameter (Seydina, 2016). After incubation, the Petri dishes are examined and the diameters of the inhibition zones surrounding the disks are measured and compared with the critical values of the various antimicrobial agents tested, to determine the clinical category (resistant, sensitive intermediary). (Williams *et al.,* 1989). Interpretation into intermediate (I) or resistant (R) sensitive (S) is carried out according to the criteria defined by (CASFM 2021).

The following tables show the antibiotics tested against *Enterobacteria*, *Pseudomonas*, and *Staphylococci* strains according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CASFM 2021).

Results and discussion

Sampling and isolation of pathogenic bacteria

32 isolates were obtained from six services in the public hospital of Medea (Algeria). The selection of services in health care is determined according to the high prevalence of nosocomial infections in these environments. The services were labeled MM: medicine man, GNC: gynecology, MS: male surgery, FS: Female surgery, ONCO: Oncology, and WM: women's medicine.

Table 1. Antibiotics tested against *Enterobacteria* isolates.

Antibiotic	Abbreviation	charge (µg)	Family
Ampicillin	AMP	10	ß- lactamine
Ticarcillin	TI	75	
Ceftriaxon	CTR	30	
Amikacin	AK	30	Aminosides
Gentamicin	HLG	120	
Tobramycin	TOB	10	
Lévofloxacin	LE	5	Fluoroquinolones
Azithromycin	AZM	15	Macrolides

The following figure shows that 70% of samples were positive compared to 30% negative. (Figure 1).

Morphological, biochemical and physiological characterization of the isolates

In current research, 5 agars medea was used to isolate pathogenic bacteria, Hektoen, Salmonella-Shigella, and Mac Conkey agar media were employed to isolate *Enterobacteria*, whereas Chapman and Cetrimide agar media were used to isolate *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains, respectively.

The colonies that appeared smooth and convex both under the naked eye and an optical microscope. Bright pink to red colonies were seen on Mac Conkey agar, indicating that *Escherichia coli* and *Klebsiella sp*. were the major isolates. This result was confirmed by yellow colonies were seen on Hektoen agar. (Figure 3).

Table 2. Antibiotics tested against *Staphylococci* isolates.

Antibiotic	Abbreviation	charge (µg)	Family
Penicillin G	P	10	ß- lactamine
Oxacillin	OX	5	
Gentamicin	HLG	120	Aminosides
Tobramycin	TOB	10	
Vancomycin	VA	30	Glycopeptides
Teicoplanin	TEC	30	
Érythromycin	E	15	Macrolides
Clindamycin	CD	2	Lincosamides
Rifampicin	RIF	30	Rifamycines
Tétracyclin	TE	30	Tétracycline
Nitrofurantoïn	NIT	100	Nitrofuranes

Table 3. Antibiotics tested against *Pseudomonas aeroginosa*.

Antibiotic	Abreviation	charge (µg)	Family
Ticarcillin	TI	75	ß- lactamine
Aztréonam	AT	30	
Amikacin	AK	30	Aminosides
Gentamicin	HLG	120	
Tobramycin	TOB	10	
Lévofloxacin	LE	5	Fluoroquinolones

The presence of *Proteus sp*. and *Shigella sp*. is indicated by the appearance of yellow colonies with black centers and wet, convex green colonies in the same medium. *Shigella* strains were also shown to be present on Salmonella Shigella medium, where they appeared as colorless colonies. In Figure 3. After incubation for 48 H at 37 °C, the strains of *Staphylococcus* that fermented the mannitol from the Chapman medium within 48 H and which caused coagulation of rabbit plasma within 24 H were identified as *Staphylococcus aureus*. (Figure 5). While the other strains were showing negative coagulation were identified as *Staphylococcus sp*. strains

Table 4. Biochemical and physiological test results of *Enterobacteria* isolates.

Tests / Strains	H2S	Gaz	Uree	Indole	Mannitol	Mobility	Citrate	glucose
E. Coli	-	+	-	+	+	+	-	+
Proteus sp	+	+	+	+	-	+	+	+
Shigella sp	-	-	-	+	-	-	-	+
Enterobacter sp	-	+	-	-	+	+	+	+
Klebsiella sp	-	+	+	-	+	-	+	+

After incubation for 48 h in Cetrimide agar, we observed the pyocyanin concentration, which results in the blue-green color. (Figure 4).

Distribution of positive samples by services

Of the 30 samples taken in the 6 services, only the ma le surgery has a rate of 100% of the positive sample.

The results are shown in Table 5.

Distribution of positive samples by sampling sites

The highest rate was observed in the beds of the patie nts 100% (06/06) followed by the samples in the surf aces of the sanitary 83% (05/06), the results are repre sented in Table 6.

Table 5. Distribution of positive samples by different services.

Services	Number of samples	Positive samples	rate
MM	5	3	60%
GNC	5	2	40%
MS	5	5	100%
FS	5	4	80%
ONCO	5	4	80%
WM	5	3	60%
Total	30	21	100%

Table 6. Distribution of samples by sampling sites.

Sampling site	Number of samples	Positif samples	Rate
soil	6	4	66%
Beds	6	6	100%
Sanitary	6	5	83%
Door handles	6	4	66%
cart	6	2	33%

Distribution of strains identified in different services.

The incidence of Gram-negative bacteria as a function of bacteria isolated from the hospital environment is 66% (21/32), and 34% (11/32) for Gram-positive, with a predominance of *Staphylococcus aureus* strains at 19% (06/32). Figure 8 shows these results.

Antibiotics susceptibility

The 32 isolates, were tested for their antibiotic sensitivity pattern against standard antibiotics. The susceptibility and resistance of the isolates were evaluated according to the following criteria: Presence or absence of the zone of inhibition, Diameter ≥ 10 mm = susceptible isolate; ≤ 10 mm = resistant isolate. The results of the antibiotic susceptibility tests carried out on the 32 isolates are shown in Figure 09, 10 and 11. According to the results of the antibiotic sensitivity, *S. aureus* is characterized by a high resistance rate of 66% (Figure 14), followed by *Enterobacter sp.* with a rate of 60% (Figure13). While *Pseudomonas aeruginosa* strains show a rate of 33%. (Figure 12).

Table 7. Distribution of resistant strains by different service.

Service	MM	GNC	MS	FS	ONCO	WM	Total
Number of isolated strains	5	2	7	4	7	3	32
Number of resistant strains	3	1	5	1	0	3	12
Rate	23%	8%	32%	8%	0%	32%	100%

MM: medicine man, GNC: gynecology, MS: male surgery,
FS: Female surgery, ONCO: Oncology, WM: women's medicine.

According to our findings, 38% (5/13) of the strains were thought to be resistant; 23% (3/13) of these strains were found in human medicine and 38% (5/13) were found in human surgery. The different antibiotic resistance rates are given in Table 7.

Discussion

Our result showed that 70% of the samples from the hospital environment were positive, of which beds and toilets had the highest rate, and 32 strains were isolated from the different surfaces of the hospital environment. This result explains that the hospital environment constitutes an important niche for multidrug-resistant bacteria that can be a reservoir from which different infections can develop (Zenati *et al.,* 2016). The emergence of antimicrobial-resistant bacteria has become a public health problem, creating a new burden on modern medical care in hospitals. (Bereket, 2012) (O'Neill, 2014). Based on morphological, biochemical identification and growth

culture on defferent agar media, a predominance of *Staphylococcus aureus* (19%) was observed, followed by *Enterobacteria* strains (*Enterobacter sp.*16%, *Proteus sp.* 16%, *Klebsiella sp.* 12.5, *Shigella sp.*9%, *E.coli* 6%) and *Pseudomonas aeruginosa* (9%). These results show the predominance of Gram-negative bacteria in nosocomial infections, but *S. aureus* was the most frequently isolated positive bacteria. These results were similar to those obtained by Rezende *et al.,* 1998.

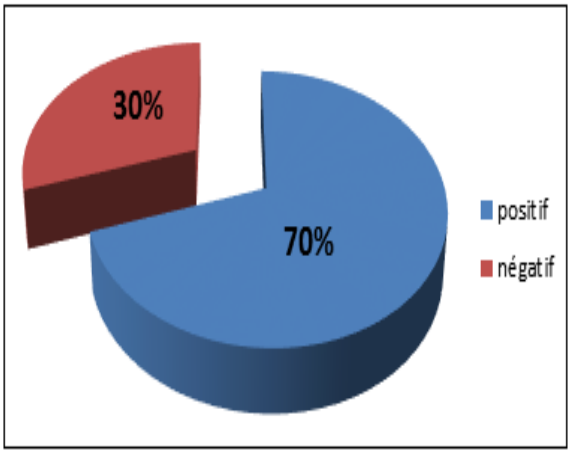


Fig. 1. Distribution of positive samples.

From primary infections, anaerobic Gram-negative bacteria were prevalent. *Enterococcus spp.* was the highest among Gram-positive aerobic bacteria followed by *Streptococcus* and *Staphylococcus* spp. *E. coli* was the predominant form among the Gram-negative aerobic bacteria followed by *K. pneumonia*, *P. aeruginosa* and *Enterobacter cloacae* (Shinagawa, 2014). *Pseudomonas aeruginosa* is known under the name of bacillus pyocyanique.

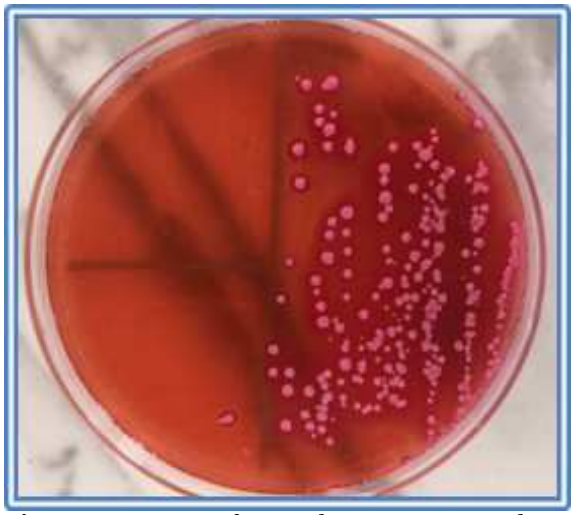


Fig. 2. Appearance of *Enterobacter* on Mac Conkey.

It is part of the groups responsible for nosocomial infections (NI). It is often present in patients with a fragile health status. The emergence of new mechanisms of resistance makes the NI to *Pseudomonas* more and more difficult to treat.

Fig. 3. Appearance of *E. Coli* on Mac Conkey.

This strain has been isolated at the level of a pipe cleaner and has kept its sensitivity to the imipenem but it has developed a resistance to Ceftazidime, Cefsulodin, and Cefepime. The resistance to beta lactams among Pseudomonas often poses serious problems because it causes the resistance to most antibiotics.

Fig. 4. Appearance of *Klebsiella*. Left: Salmonella Shigella medium; right on Hektoen.

The resistance developed by Pseudomonas is usually associated with mutations leading to a hyper

expression of the chromosomal class C beta-lactamase.

Fig. 5. Macroscopic appearance of *Pseudomonas aeruginosa* on cetrimide agar.

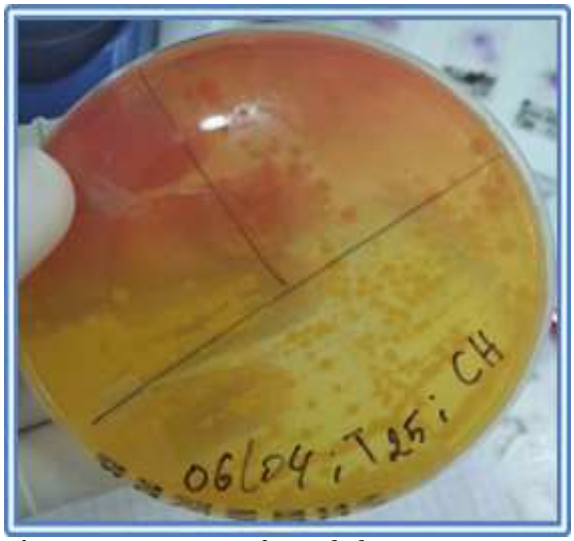


Fig. 6. Appearance of *Staphylococcus aureus* on Chapman.

According to results obtained by positive samples of sampling sites, the highest rate was observed in the beds of the patients, this result was similar to this obtained by Rajaa Amiyare *et al.,* 2015. According to our opinion, the high concentration of microbial populations in the hospital environment, the distribution of the same types of strains across the service, the severity of patients' pathologies, the presence of elderly people, and the rise in staff numbers are all potential vectors of nosocomial infections (doctors, nurses, and trainees).

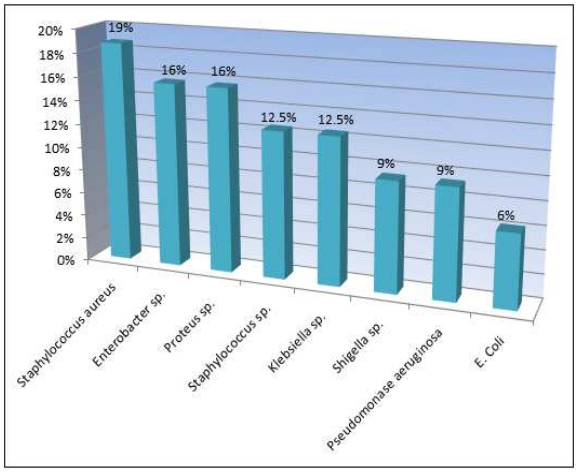


Fig. 7. Distribution of strains identified in different service.

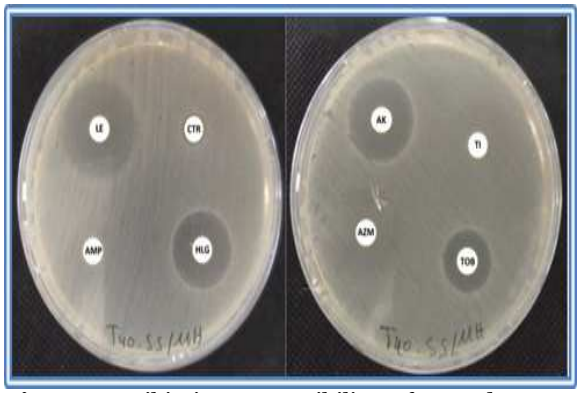


Fig. 8. Antibiotics susceptibility of *Pseudomonas aeruginosa* isolates.

According to our study, the highest rate of resistant strains was observed in the male surgery (MS) and women's medicine (WM) service, this result differs from this obtained by Rezende *et al.,* 1998, who found that cardiac surgical service had high prevalence of nosocomial infections followed by pediatrics surgery.

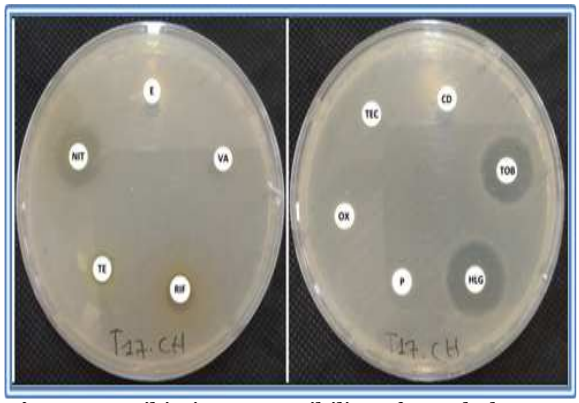


Fig. 9. Antibiotics susceptibility of *Staphylococcus aureus* isolates.

The surfaces in hospitals is highly contaminated. It's critical to know how many species cause nosocomial infections. 32 strains from 7 highly pathogenic species may suggest that aseptic technique, hygienic regulations, manual or automatic cleaning, and disinfection processes, and/or staff hand hygiene are not being followed. In fact, poor cleanliness is the primary contributor to hospital infections. (Chaplain, 1997).

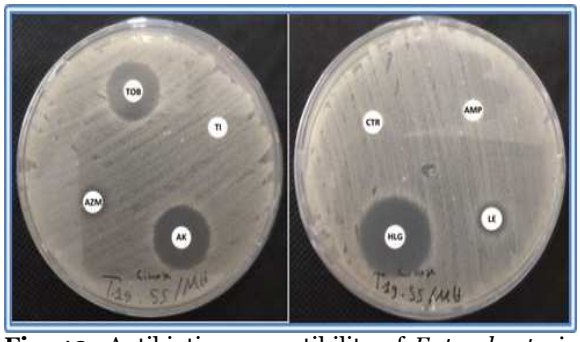


Fig. 10. Antibiotics susceptibility of *Enterobacteria sp*. isolates.

According to (Chouki *et al.,* 2004). The resistance to antibiotics of the B-Lactam family, especially penicillins is moderate with a rate of 63.63%, this result is close to our values found in relation to *Enterobacteria* (66.73) but concerning *Staphylococci* such as *S. aureus*, the resistance is 100% for antibiotics of the β-lactam family: Penicillin G (100%) and oxacillin (100%). β-Lactam resistance is due to inactivation of β-lactamases.

Attempts to identify inhibitors of common b-lactamases began in the mid-1970s, triggered by the appearance of the transferable TEM-1 penicillinase in Neisseria gonorrhoeae (Ashford *et al.,* 1976) and Haemophilus influenzae (Gunn *et al.,* 1974; Khan *et al.,* 1974). As the result of natural product screening, clavulanic acid with a novel clavam structure was identified as a broad spectrum inhibitor of the staphylycoccal penicillinases and most of the recognized plasmid-encoded penicillinases found in enteric bacteria (Reading and Cole 1977; Cole 1982), including the highly prevalent TEM and SHV enzymes (Simpson *et al.,* 1980).

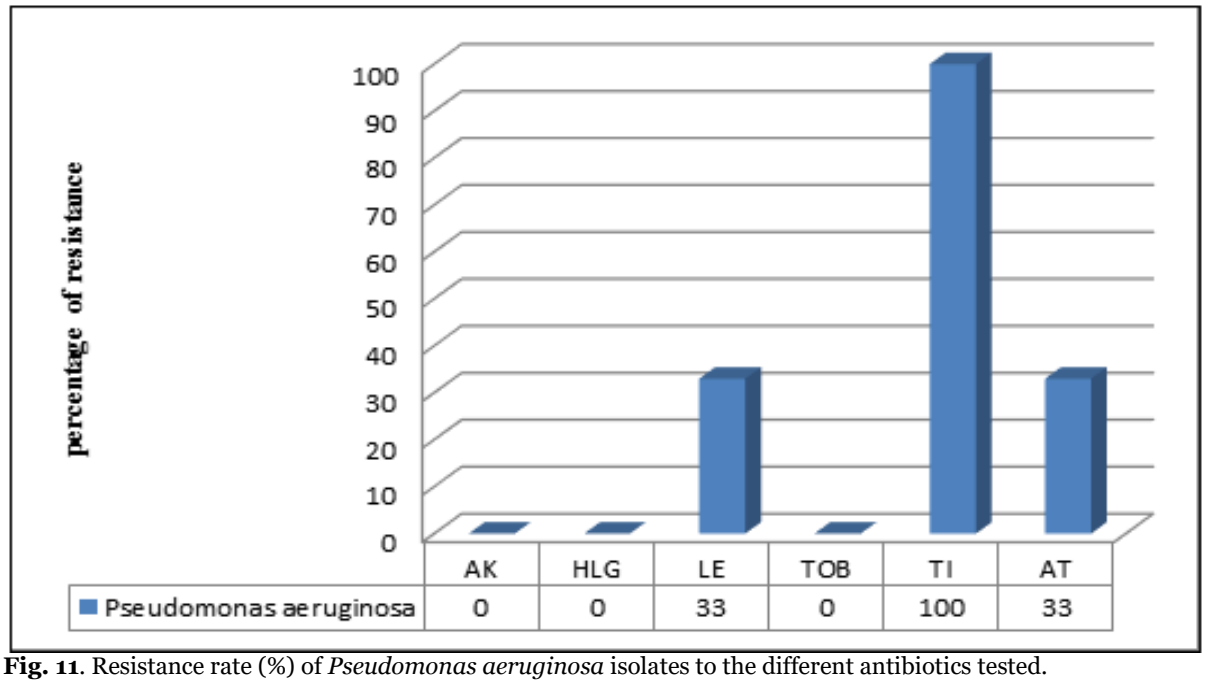
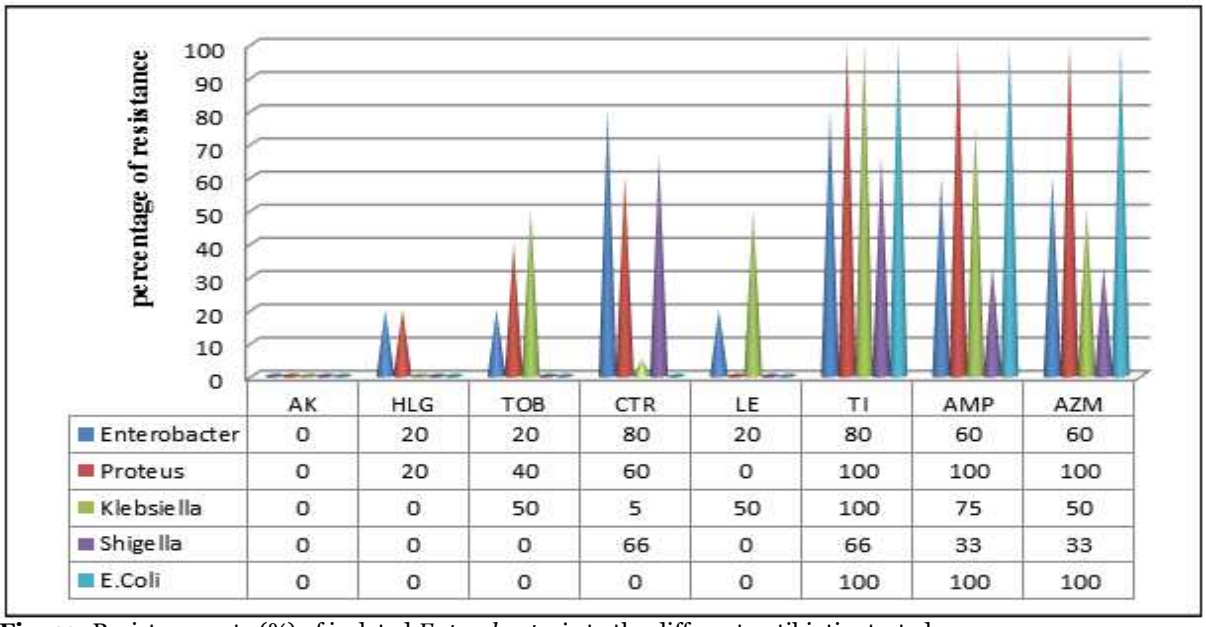


Fig. 11. Resistance rate (%) of *Pseudomonas aeruginosa* isolates to the different antibiotics tested.

The TEM blactamase was shown to be inactivated by this suicide inhibitor that initially acylates the active site serine with transient inhibition that includes hydrolysis of the inhibitor before complete enzyme inactivation (Charnas *et al.,* 1978; Charnas and Knowles 1981). Aminoglycoside resistance was very low in *Enterobacteria* and *Staphylococci* with resistance rates of (7.76) and (37.5) respectively,

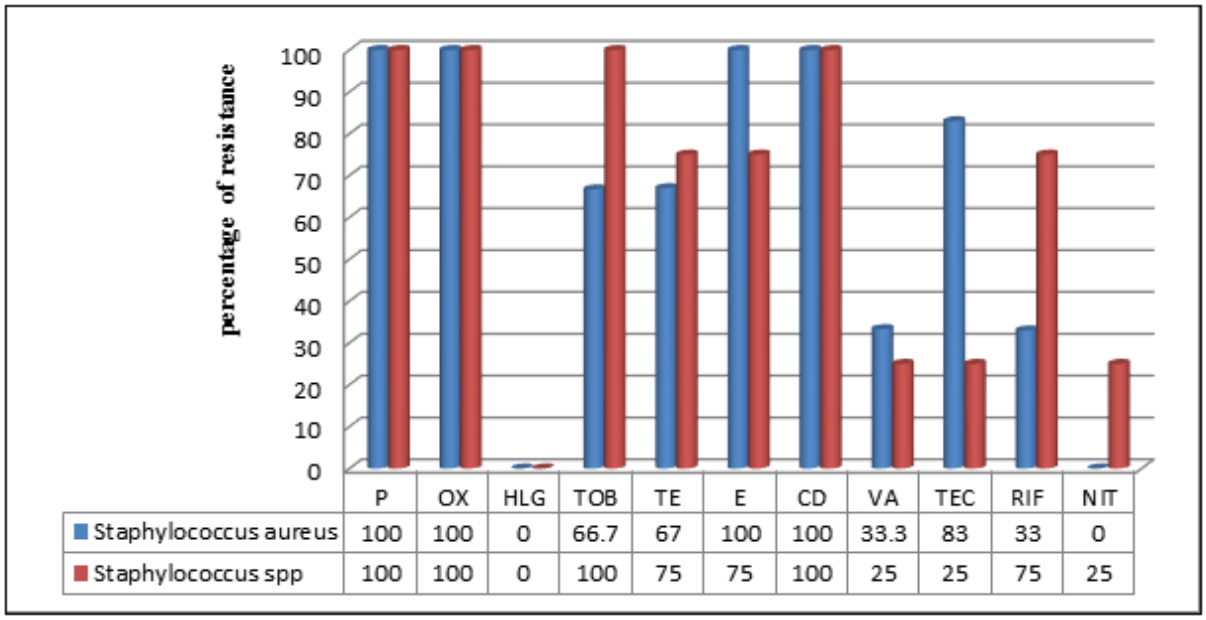
similar to that founed by (Tomasz *et al.,* 2008). The resistance of *S. aureus* to aminoglycosides can be ensured by 2 mechanisms. The first one consists of chromosomal mutation affecting ribosomes may confer a high level of resistance to the streptidine-containing aminoglycoside streptomycin (Lacey & Chopra 1972), the second mechanism results from mutations affecting the permeability of the cellular permeability may provide low-level cross resistance to most aminoglycosides (Shannon & Phillips 1982; Lyon & Skurray 1987), and the third one is ensured by the production of enzymes that inactivate the antibiotic, such as aminoglycoside acetyltransferases (AAC), aminoglycoside adenyltransferases (AAD) and aminoglycoside phosphotransferases (APH) (Foster 1983; Lyon & Skurray 1987; Shaw *et al.*,1993).

For the 3 strains of *P. aeruginosa*, we observed a low resistance 27.66 compared to resistance rates reported in Algeria, 38% at the CHU of Oran (Sefraoui, 2015).



	AK	HLG	TOB	CTR	LE	TI	AMP	AZM
Enterobacter	0	20	20	80	20	80	60	60
Proteus	0	20	40	60	0	100	100	100
Klebsiella	0	0	50	5	50	100	75	50
Shigella	0	0	0	66	0	66	33	33
E.Coli	0	0	0	0	0	100	100	100

Fig. 12. Resistance rate (%) of isolated *Enterobacteria* to the different antibiotics tested.



	P	OX	HLG	TOB	TE	E	CD	VA	TEC	RIF	NIT
Staphylococcus aureus	100	100	0	66.7	67	100	100	33.3	83	33	0
Staphylococcus spp	100	100	0	100	75	75	100	25	25	75	25

Fig. 13. Resistance rate (%) of Staphylococcal strains isolated to the different antibiotics tested.

This wide spread of *Enterobacteria*, *S. aureus* and *P. aeruginosa* in hospital settings is accompanied by an increase in antibiotic resistance. This makes the severity of nosocomial infections and declares the state of alarm in hospitals. The selection pressure related to the use of broad-spectrum antibiotics and the existence even in the hospital environment of a genetic support, are important factors in the evolution of antibiotic resistance. According to Hancock and Speert (2000), *Pseudomonas aeruginosa* exhibits resistance to a number of antibiotics, including aminoglycosides, quinolones, and -lactams. The main mechanisms employed by *P. aeruginosa* against antibiotic attack can generally be divided into three categories: intrinsic, acquired, and adaptive resistance. Low permeability of the outer membrane, the expression of efflux pumps that expel antibiotics from the cell, and the production of enzymes that inactivate antibiotics are all characteristics of *P. aeruginosa's* intrinsic resistance. *P. aeruginosa* can develop resistance either through mutations or horizontal transfer of resistance genes (Breidenstein *et al.,* 2011). For the last few decades, hospitals have taken the hospital-acquired infections seriously. Several hospitals have established infection tracking and surveillance systems in place, along with robust prevention strategies to reduce the rate of hospital-acquired infections. (Habboush *et al.,* 2022).

Conclusion

Our study is based on samples from the hospital environment. A total of 32 strains were isolated and identified, with a predominance of gram-negative bacilli at 66% and gram-positive cocci at 34%, with a predominance of *Staphylococcus aureus* strains at 19%. We also determined the sensitivity of the isolated bacterial strains to the different families of antibiotics, of which half of the strains are resistant (50%). (16/32), particularly antibiotic resistance can serve as reservoirs for potentially pathogenic microorganisms that are difficult to eradicate, the cleaning and disinfection of environmental surfaces are essential for reducing the incidence of nosocomial infections. However, it is worthwhile to continue the research to take samples from the same sites for re-identification to evaluate the hygiene measures in place to eliminate these germs.

Acknowledgments

We would like to express gratitude to the department of natural and life sciences, Faculty of Sciences, Yahia-Fares University, Medea, Algeria, Saidal antibiotical group and public hospital staff in Medea, Algeria for supporting the study and collection of samples.

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