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Research Article

Study of Colonization by Antibiotic-Resistant Staphylococci in Hospital Liquid Waste in the Southern Region of Sidi-Bel-Abbès

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ABSTRACT

The problem of hospital effluent discharges is increasingly important, as the drug-induced anthropization of hospital wastewater favors the emergence and dissemination in the environment of antibiotic-resistant microorganisms. The present study is part of the characterization of the multi-resistance to antibiotics of Staphylococcus spp. isolated from the effluents of a health establishment. For this purpose, 30 samples of wastewater were collected from the dialysis machines in the nephrology department of the Public Hospital Establishment of Telagh. Isolation is performed on Chapman medium and identification of the strains is performed by the API Staph gallery. The isolated strains were subjected to a standard antibiogram according to the recommendations of the Institute of Clinical and Laboratory Standards for different families of antibiotics (β-lactams, aminoglycosides, fluoroquinolones, and sulfonamides). Biochemical identification showed that our isolates were non-*S. aureus* strains, normally considered coagulase-negative, were found to be coagulase positive. Our results indicated that there is a passage of multidrug-resistant staphylococci in the environment, which requires periodic monitoring of staphylococci resistance to antimicrobials to control their spread.

INTRODUCTION

Water is an essential natural resource on earth, both for human survival and health. In addition, it presents a benefit for agricultural, industrial and tourist activities [1]. Nowadays, aquatic environments deserve special attention, given that they are highly altered and seriously threatened by anthropogenic activities [2]. Many studies have shown the role of the environment as a source or reservoir of antibiotic-resistant bacteria and antibiotic residues that contribute to the selection of multi-resistant germs [3]. In this context, the problem of hospital effluent discharges is increasingly important. The latter produce large volumes of liquid effluents [4]. For each patient, the hospital consumes about one cubic meter of water, depending on the type of activity.

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Healthcare facilities generate liquid waste that is a reservoir of potentially dangerous microorganisms such as Staphylococcus, and hospital effluents are 5 to 15 times more ecotoxic than urban effluents [5]. Nevertheless, the vast majority of hospitals do not have a system of treatment and purification of liquid effluents, which in most cases are discharged into the environment and large lakes [6]. As the anthropization of waste water by drugs, favors the emergence and diffusion in the environment of antibiotic-resistant microorganisms. The present study is part of the characterization of multiresistant antibiotics, of Staphylococcus spp isolated from the effluents of a health care facility. Our objective is to evaluate, for the first time to our knowledge, the bacteriological quality of the effluents of the Public Hospital Establishment (EPH) of the city of Telagh, by the determination of the degree of pollution of these effluents by the Staphylococci multi-resistant to antibiotics.

MATERIALS AND METHODS

Nature of the Samples

We took the sample at the nephrology and hemodialysis department at the EPH of Telaghof the liquid waste of the generators of the dialysis machines. The collection was in conditions that avoid any accidental microbial contamination [7]. For each collection point, the valve was opened, and the water was allowed to flow for 2 minutes before taking the sample. The collected sample was taken to the laboratory the same day in a glacier for analysis.

Bacteriological Analysis

The methods used in the search and identification of germs present in the liquid waste of the dialysis machine are routine methods described by [8]. Isolation is performed on the BHI medium, from the liquid waste of the dialysis machine. Incubation is performed at 37°C for 24 hours. After a morphological reading, the different suspected colonies are re-isolated on the Chapman medium to obtain pure cultures. A first orientation was performed after verification of the absence of contamination by Gram staining and catalase and oxidase tests. The identification of the genus is performed by the appearance of colonies on agar and mannitol fermentation on Chapman medium. The Staphylococci thus appear as cockles, Gram + and catalase+. The presumptive colonies were confirmed by biochemical characterization using the Bio Merieux API-20-Staph system [9]. After the phenotypic identification of the strains, different virulence factors were searched, namely free coagulase, and deoxyribonuclease (Dnase) [10]. The latex agglutination test on the card for the search for clumping factor, protein A and capsular polysaccharides of *S. aureus* was used by the Staphytect Plus kit (Oxoid, Basingstoke, United Kingdom) [11].

Antibiotic Susceptibility Testing: Diffusion Antibiogram Method

Agar diffusion susceptibility testing consists of depositing disks impregnated with a known concentration of antibiotics on an agar plate inoculated with the bacteria to be tested [12]. The susceptibility test is a tool to assist in antibiotic policy through its results; it collects data on good practices in the use of antibiotics and identifies the global or specific ecology of populations [13]. Susceptibility testing was performed by disc (Bio-Rad, France) and judged by the Clinical and Laboratory Standards Institute [14].

Staphylococcus spp. strains were inspected for their susceptibility behavior against various antimicrobial agents used in veterinary and human medical practices. The antibiotics tested were Penicillin G (PG-10 IU), Amoxicillin + clavulanic acid (AMC- 20/10 µg), Oxacillin (OX- 1 µg), Cefoxitin (Fox-30 µg), Erythromycin (E-15 µg), Neomycin (N- 30 µg). Enrofloxacin (ENR-5 µg), Trimethoprim + sulfamethoxazole (SXT-1.25 / 23.75 µg), Tetracycline (Te -30 µg), Vancomycin (VA-30 µg), Bacitracin (Ba-130 µg), Clindamycin (Cm-2 µg). The following reference strains were used for quality control of each antibiogram: *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922.

Investigation of Biofilm Production by the Congo Red Agar Method:

Congo Red Agar is a very suitable medium for the detection of slime-producing strains. On this medium strains expressing PIA (Polysaccharide Intercellular Adhesion) give black colonies with rough surfaces against red-colored colonies with smooth surfaces for PIA negative strains [15]. The medium was prepared with 37 g/L BHIB, 50 g/L sucrose,10g/L agar and 0.8 g/L Congo Red and autoclaved at 121°C for 15 minutes [16].

RESULTS AND DISCUSSION

Water in hospitals can be contaminated by numerous multi-resistant bacteria and is a preferred vector for many bacterial pathologies, which can lead to infections in fragile or immune-compromised patients [17]. In the present study, antibiotics are chosen for the antibiogram taking into account the most frequently used drugs in human medicine in Algeria. This study was conducted to study the resistance of Staphylococcus spp. strains, isolated from hospital liquid discharge to 12 antibiotics of different families. Of the 30 samples taken, 09 were positive (bacterial development), i.e. a rate of 30%, and of a total of 17 strains isolated from 09 samples, 03 met the macroscopic and microscopic characteristics of the genus Staphylococcus, i.e. a rate of 18%.

Identification by biochemical galleries revealed three different biotypes: 7334110: *Staphylococcus cohniisppcohnii*, 6204100: *Staphylococcus cohniisppcohnii* and 4100112: *Staphylococcus capitis*. According to Table 1, multiple antibiotic resistances were encountered in 03 strains of the isolated staphylococci.

Table 1: Multiple antibiotic resistance among isolated staphylococcal strains

The biotypes	Resistant strains	Number of resistant strains compared to the number of antibiotics			
		1	2	3	4
7334110	1			x	
6204100	1		x		
4100112	1			x	

In literature, multiple resistance means: "resistance to more than one antimicrobial agent", but no standardized definition for multidrug resistance has yet been agreed upon by the medical community [18]. At the end of the antibiotic resistance tests, the results showed heterogeneity for all antibiotics, the highest resistance rates were recorded with Penicillin (100%) and oxacillin (100%), these results are very close to those reported by Touzani et al [1] with a resistance rate to penicillin and oxacillin of 100% and Allioua [19] who found a resistance rate to penicillin of 100% (Table 2).

Table 2: Resistance and sensitivity of Staphylococcus strain to different antibiotics.

Antibiotics Tested	Code	Disc Load	Critical Diameters (mm)			Biotypes		
			R	I	S	7334110	6204100	4100112
Pénicilline	PG	10 UI	≤28	-	≥29	R	R	R
Amoxicilline+ Acide Clavulanique	AMC	20/10 µg	≤19	-	≥20	S	S	S
Oxacilline S. aureus S.C.N	OX	1 µg	≤10 ≤17	11-12	≥13 ≥18	R	R	R
Céfoxitine**	FOX	30 µg	≤21	-	≥22	S	S	R
Erythromycine	E	15 µg	≤13	14-22	≥23	I	S	S
Néomycine	N	30 µg	≤13	14-17	≥18	S	S	S
Enrofloxacine	ENR	5 µg	≤16	17-22	≥23	S	I	S
Triméthoprime+ Sulfaméthoxazole	SXT	1.25/23.75 µg	≤10	11-15	≥16	S	S	S
Tétracyclines	Te	30 µg	≤14	15-18	≥19	R	S	S
Vancomycine**	Va	30 µg	-	-	≥15	S	S	S
Bacitracine	B	130 µg	<15	-	≥15	S	S	S
Clindamycine	Cm	2 µg	<14	15-20	≥21	I	S	S

The diffusion of MRSA in hospitals or community settings poses a public health problem, requiring the determination and understanding of the characteristics of resistance to antibiotics, which represent one of the essential goals of medical bacteriology, being able to evolve therapeutic strategies [20]. The global analysis of resistance of MRSA to antibiotics confirms the multi-resistance of these germs that are known for their ability to resist several other families of antibiotics [21].

In the present study, two out of three strains of the Staphylococci isolated from the samples taken showed a positive phenotype for coagulase production. The presence of coagulase in the isolated strains is used to distinguish between coagulase-positive staphylococci mainly *S. aureus*, as well as *S. intermedius*, *S. hyicus* and coagulase-negative staphylococci [22]. All three staphylococcal strains isolated reveal a positive Dnase. DNase-positive microorganisms are surrounded by clear areas of depolymerized DNA, while the parts of the medium further away from the seeding strip are opaque and whitish, due to the effect of polymerized DNA [23]. It is an important criterion used to distinguish between pathogenic staphylococci and non-pathogenic resident flora [24]. It has been reported that coagulase-negative Staphylococci may also have Dnase activity [25]. In the present study, out of 03 staphylococcal isolates, 03 (100%) were found to be coagulase-producing strains bound. A positive result is considered if there is an agglutination of blue latex particles in less than 20 seconds in the probable presence of *S. aureus*. However, other

species such as *S. hyricus* and *S. intermedius* can be coagulase-positive [26]. The Staphytect TM Plus Latex Agglutination Test consists of blue latex particles, sensitized with fibrinogen and IgG containing specific antibodies to *S. aureus* capsular polysaccharides, that agglutinate within 20 seconds. aureus, agglutinating in the presence of Clumping Factor (extracellular coagulase) and/or protein (A) carried by certain staphylococci (See Fig. 1). Agglutination occurs mainly in the presence of *S. aureus*. Nevertheless, other species such as *S. hyricus* and *S. intermedius* can be coagulase-positive [27].

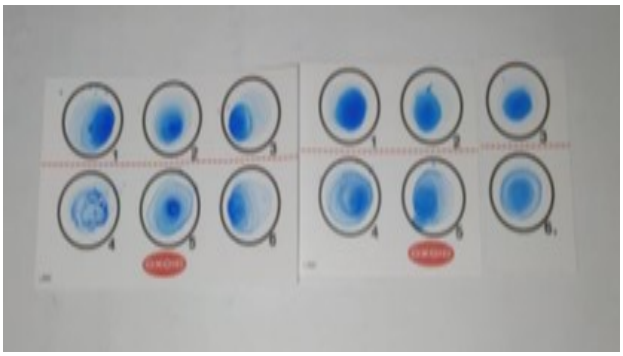

Figure 1: Staphytect Plus Test Result

Several researchers have studied the strategies employed by microorganisms to produce biofilms. They have shown that biofilm-producing bacteria secrete certain chemicals that protect them from disinfectants, anti-microbial agents and host immune systems [28]. Biofilm-producing bacteria are responsible for many infections that are difficult to eradicate. They show resistance to antibiotics, through various methods such as limited penetration of the antibiotic into the biofilms [19]. The present study used a qualitative method that is based on the phenotypic character of strains seeded on Congo Red medium. The results obtained allowed us to observe that the 3 strains of Staphylococci produce a slime and show a positive phenotype in the medium, by colonies with a black center and red outline (Fig. 2).

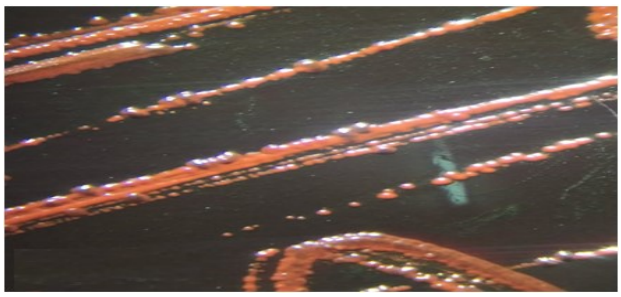

Figure 2: Result of biofilm production on Congo Red agar

Using Congo Red Agar:

The congo red dye interacts directly with certain bacterial polysaccharides forming a slime and resulting in black colonies on CRA medium in contrast to non-producing colonies [29]. Microorganisms growing in a biofilm are intrinsically more resistant to antimicrobial agents than planktonic cells [30], which confirms the results we obtained before concerning the tested antibiotics. High concentrations of antimicrobial agents are necessary to inactivate them, as the resistance to antibiotics can increase by 1000 times [31].

CONCLUSION

The results obtained show that the wastewater from the EPH of Telagh is a reservoir of pathogenic and multi-resistant Staphylococci. These bacteria present resistance to two or three families of antibiotics. The failure to treat these effluents and the lack of basic sanitation services may contribute to the establishment of pathways for the dissemination of these microorganisms and their resistance genes in the environment, which requires monitoring hospital effluents and adopting measures to prevent this dissemination. In the perspective of this work, it would be important to enlarge the sample size to be studied to have a better epidemiological appreciation and to identify in-depth at the genetic levels the multiresistance to antibiotics as virulence factors of the Staphylococcus strains in the hospital effluents of the Wilaya of Sidi-Bel-Abbès.

REFERENCES

[1] Touzani I, Machkor M, Boudouch O., ElMachrafi I, Flouchi R and Fikri-Benbrahim K. Evaluation of physicochemical and bacteriological parameters of effluents in TAZA hospital-morocco-application of principal component analysis (PCA). PollRes. 2020; 39 (4): 872-878.

[2] Édouard R. Évaluation des impacts des eaux industrielles sur la rivière trou-du-nord. Institut des sciences, des technologies et des études avancées d'haïti. 2021; Mémoire du diplôme de maîtrise en science.

[3] Koudokpon H, Dougnon V, Lougbegnon C, Deguenon E, Mousse W, Jerrold A et al. 2021. Antibiotic Resistance Profile and Resistance Determination of Bacteria Isolated from Water in Southern Benin.

Journal of Advances in Microbiology. 2021; 21(4): 91-105: 91-105.

[4] Smyth C, O'Flaherty A, Walsh F and Do TT. Antibiotic-resistant and extended-spectrum β-lactamase producing fecal coliforms in wastewater treatment plant effluent. Environmental Pollution. 2020; 262: 114244.

[5] Todedji JN, Degbey CC, Soclo E, Yessoufou A, Goudjo F, Hounfodji JW et al. Caractérisation physico-chimique et toxicologique des effluents des Centres Hospitaliers et Universitaires du département du Littoral du Bénin. International Journal of Biological and Chemical Sciences. 2020; 14(3):1118-1132.

[6] Todedji J, Degbey C, Soclo E, Yessoufou A, Hounfodji JW, Goudjo F et al. Microbiological Quality of the Effluents Produced by the University and Hospital Centres in the Department of Littoral, Republic of Benin. Open Journal of Epidemiology. 2020; 10 (01):66.

[7] Rodier J, Legube B and Merlet N. L'analyse de l'eau: Méthodes générales de prélèvement, transport et conservation, 9th ed. Paris France: Dunod; 2009. 721-728.

[8] Carl AB and Pradip P. Staphylococcus. In: encyclopedia of food microbiology, 2th ed. London UK: Academic Press; 2014. 482-501.

[9] Randriamampionona E. Identification et caractérisation des Staphylocoques à Coagulase Négative résistants à la méthicilline isolés d'échantillons sanguins de patients fébriles dans les centres de santé de la région Analamanga-Antananarivo. 2018; Thèse de doctorat d'université d'Antananarivo.

[10] Denis F, Ploy MC, Martin C, Bingen E and Quentin R. Cocci à Gram positif. In Bactériologie médicale: techniques usuelles. 2th ed. Paris: Elsevier Masson; 2011. 287-299.

[11] Soares JC, Marques MR, Tavaria FK, Pereira JO, Malcata FX and Pintado MM. Biodiversity and characterization of Staphylococcus species isolated from a small manufacturing dairy plant in Portugal. International journal of food microbiology. 2011; 146(2): 123-129.

[12] Quentin-Noury C. Automatisation de l'antibiogramme au laboratoire debactériologie. Revue Francophone des Laboratoires. 2016; 2016(482): 49-59.

[13] Caron F. L'antibiogramme : un quadruple outil pour le clinicien. Journal des Antiinfectieux. 2012; 14(4): 168-174.

[14] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; Approved standard, 3th ed. USA: Wayne PA; 2008. M31-A3.

[15] Nasr SA, Abu Shady HM and Hussein HS. Biofilm formation and presence of ica AD gene in clinical isolates of staphylococci. The Egyptian Journal of Medical Human Genetics. 2012; 13(3): 269-274.

[16] Jain A and Agarwal A. Biofilm production, a marker of pathogenic potential of colonizing and commensal staphylococci. Journal of Microbiology and Methods. 2009; 76(1): 88–92.

[17] BoillotC. Évaluation des risques écotoxicologiquesliés aux rejets d'effluents hospitaliers dans les milieux aquatiques: Contribution à l'amélioration de la phase « caractérisation des effets. 2008; Thèse de doctorat, Institut National des Sciences Appliquées de Lyon.

[18] Magiorakos AP, Srinivasan A, Carey R, Carmeli Y, Falagas M, Giske Cet al. Multidrug-resistant, extensively drug-resistant and pan drug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection. 2012;18(3): 268-281.

[19] Allioua A. Les staphylocoques: sensibilité aux antibiotiques et profil moléculaire de Staphylococcus aureus résistants à la méticilline. 2015; Thèse de doctorat en microbiologie appliquée. de la Faculté des sciences Annaba.

[20] Dumitrescu O, Dauwalder O, Boisset S, Reverdy MÉ, Tristan A and Vandenesch F. Résistance aux antibiotiques chez Staphylococcus aureus. Médecine/sciences. 2010; 26(11), 943–949.

[21] Thibaut S, Coeffic T, Boutoille D, Lemenand, O., Birgand, G., Caillon, J et al. Phénotypes de résistance aux antibiotiques de Staphylococcus aureus isolées d'hémoculture en soins de ville. Médecine et Maladies Infectieuses. 2020; 50(6), S35.

[22] Riegel P, Jesel-Morel L, Laventie B, Boisset S, Vandenesch F and Prévost G. Coagulase-positive Staphylococcus pseud intermedius from animals causing humanen docarditis.

International Journal of Medical Microbiology. 2011; 301(3): 237-239.

[23] Sandel MK and Mc Killip JL. Virulence and recovery of Staphylococcus aureus relevant to the food industry using improvements on traditional approaches. Food Control. 2004; 15(1): 5-10.

[24] Boerlin P, Kuhnert P, Hüssy D, and Schaellibaum M. Methods for identification of Staphylococcus aureus isolates in cases of bovine mastitis. Journal of clinical microbiology. 2003, 41(2): 767-771.

[25] Markey B, Leonard F, Archambault M, Cullinane A and Maguire D. Clinical veterinary microbiology. 2th ed. China: Elsevier Health Sciences; 2013.105-433.

[26] Murray PR, Baron EJ, Jorgensen JH, Landry ML, and P faller MA. Staphylococcus, Micrococcus, and Other Catalase-Positive Cocci. In: Manual of Clinical Microbiology.9th ed. Washington DC: ASM Press; 2007. 390-411.

[27] Devriese LA, Vancanneyt M, Baele M, Vaneechoutte M, De Graef E., Snauwaert C., et al. Staphylococcus pseudintermedius sp. nov., a coagulase-positive species from animals. International Journal of Systematic and Evolutionary Microbiology. 2005, 55(4): 1569-1573.

[28] Flemming HC, Baveye P, Neu TR, Stoodley P, Szewzyk U, Wingender J et al. Who put the film in biofilm? The migration of a term from wastewater engineering to medicine and beyond. npj Biofilms and Microbiomes. 2021, 7(1):1-5.

[29] Oliveira TS, Al Aukidy M and Verlicchi P. Occurrence of Common Pollutants and Pharmaceuticals in Hospital Effluents. In: The Handbook of Environmental Chemistry; Verlicchi, P. Springer: 2017. 17–32.

[30] Varela MF. Antimicrobial efflux pumps. In Antibiotic Drug Resistance; Capelo-Martínez JL, Igrejas G. Hoboken, NJ, USA: John Wiley & Sons, Inc; 2020. 167–179.

[31] Simoes M. Antimicrobial Strategies Effective Against Infectious Bacterial Biofilms. Current Medicinal Chemistry. 2011, 18(14), 2129–2145.