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Research Article

Monitoring the Evolution of *Pseudomonas aeruginosa* Resistance against Routine Antibacterial Agents

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ABSTRACT

Bacterial resistance to antibacterial is a major public health problem. To contribute to the monitoring of multidrug resistance of *Pseudomonas aeruginosa,* we have tested 356 strains. These were isolated from pathological products of outpatient or inpatient. These strains were identified by the highlighted specific pigments and gallery API 20NE then submitted to several sensitivity tests against antibacterial agents. The latter was evaluated by the diffusion method on agar medium. For chlorinated water which is the most widely used disinfectant worldwide, we sought the lethal concentrations by liquid-based diffusion method. The results demonstrated that among the amino glycoside only amikacin showed significant efficacy. Among beta-lactam, the imipenem and piperacillin are the most effective relative to other antibiotics tested. Among the antiseptics tested only hydrogen peroxide at 10 volumes demonstrated efficacy compared with iodine alcohol at 10% and surgical alcohol at 70°. Strains tested showed a tolerance at concentrations at least most important chlorinated water at 13 °. The latter has a static effect only up to 2%.

Keywords: Multiresistant, *Pseudomonas aeruginosa*, antibiotics, antiseptics, disinfectant.

INTRODUCTION

Bacterial resistance to antimicrobial agents is a growing problem in medical practice. As to the discovery of new antibiotics[1], bacteria have gradually accumulated in their genetic material multi resistance genes[2,3], the latter constitutes a major public health problem[4]. *Pseudomonas aeruginosa* pyocyanique bacilli) is an opportunistic bacterium, able to adapt in any environments[5-9]. This species has a natural resistance to many antibiotics over time, the strains have developed acquired resistance to many antibiotics[6]. It is able to acquire new strengths against usually active compounds. It differs from other species occurring in the hospital by its relatively high pathogenicity against immune compromised patients (diabetes, cystic fibrosis, cancer, HIV, mechanical ventilation) and its cross resistance to many antibiotics of different families[5,6]. The severity and mortality observed in the bacterium *Pseudomonas aeruginosa* infections are due to its intrinsic properties, its ability to adapt between the ability of others to acquire numerous mechanisms of resistance to many antibiotics and antiseptics[8, 10, 11]. The most disturbing fact of recent years is reporting more and more common *P. aeruginosa* strains resistant to an increasing number of antibiotics. In some cases, strains show totally resistant to all available molecules. This bacterium is a disease "problem", especially as very few new drugs are marketed by the pharmaceutical industry. Thus, several authors have sounded the alarm facing the imminent era of "post-antibiotic"[10, 11]. The emergence and dissemination of new mutants of *P. aeruginosa*are all factors that can lead to treatment[12]. To face this challenge, health facilities should establish monitoring and control strategies. These actions involve multidisciplinary collaboration involving infectious disease specialists, microbiologists, hygienists and epidemiologists[13]. To contribute to the monitoring of multiresistant strains of this species. We focus in this letter, on the determination of the bacterial strains sensitivity profiles of *P. aeruginosa* isolated from different origins related to antibiotics (beta-lactam and aminoglycoside antibiotics), determination of effectiveness of commonly used antiseptics and search most used effective disinfectant concentrations (chlorinated water) for cleaning the premises.

MATERIAL AND METHODS

Collection and transportation of strains

Pseudomonas aeruginosa strains tested in this study were collected from various medical bacteriology services, hospitals, clinics or private laboratory. We have been tested 356 strains multiresistant whose origins differ according to age, sex and type of sample patients of different categories. The strains tested were transported to the laboratory in conservation agar tubes.

Identification

Identification of *P. aeruginosa* was previously conducted based on phenotypical and morphological criteria (colony morphology, pigmentation, lactose fermentation, oxidase and catalase activity)[14-16]. Specific pigments pyocyanin and pyoverdin were detected by

using King medium A and King medium B[16].The most interesting strains are identified by API 20NE kit biomerieux[17,18].

Determination of the sensitivity profiles against antimicrobial

Antimicrobial susceptibility tests were performed according to Clinical and Laboratory Standards Institute (CLSI)[19].

Antibiogram

The resistance profile of the strains isolated for antibiotics was determined by the diffusion method on agar medium. The antibiotics used in this study were selected basically on their spectrum of activity that extends worm species studied. This was performed by the Kirby-Bauer disc diffusion method, which was performed using the Clinical Laboratory Standards International guidelines [19].

Antiseptogram

This research aims to determine the susceptibility of isolated strains to antiseptics commonly used to disinfect the skin. Three antiseptics (surgical alcohol 70, iodine 10% alcohol and 10 volume hydrogen peroxide) were tested in this study. The method is applied by analogy to the diffusion susceptibility testing method on agar medium[19]. Using sterile forceps bacteriological; apply soaked disks with antiseptic to the surface of Muller Hinton agar seeded beforehand. Press each one of them to ensure its implementation. Once applied, the disk should not be moved and incubate 18 hours at 35 ° C. The diameters of the inhibition zones are accurately measured using a metallic caliper rule, outside of the closed box.

Determining inhibitory concentrations of chlorinated water

The method is applied by analogy to the method for determining minimum inhibitory concentrations bactericidal and bacteriostatic antibiotics by Broth Dilution Susceptibility Tests, [20, 21].

They were determined according to Clinical and Laboratory Standards Institute (CLSI) guidelines[22]. Prepare a series of different concentrations of chlorinated water at 13 ° in a Muller Hinton broth in test tubes in an amount of 5 ml per tube. Inoculate each tube with 500µl of the bacterial suspension equivalent to 0.5 McFarland opacity. After 18 hours incubation at 35°C; tubes with a concentration = disorder is not inhibitory, those not having a disorder = inhibitory concentration. To confirm the inhibition is static or bactericidal we reseeded negative tubes on agar box by longitudinal striated with four strains per dish.

RESULTS AND DISCUSSION

Antibiogram

The results of the susceptibility testing show that the tested strains have varying susceptibility profiles (Figure 1). All tested strains are resistant to penicillin novobiocin; PNV and lincomycin; L (R + I = 100%)). 90% of strains are resistant to amoxicillin + clavulanic acid (AMC) and 66.7% to kanamycin (K). The rate of resistance to ceftazidime (CAZ), aztreonam (TMJ), ticarcillin (ICT) and colistin (CT) are varied between 26.6%) and 20%.

However, it was noted that only 13.3% of the tested strains were resistant to gentamicin (CN) and only 10% to tobramycin (TOB). Too low resistance (3.3%)) was observed for imipenem (IPM) and piperacillin (PRL). The Amikacin (AK) showed a greater efficiency, where no strain has shown resistance to this antibiotic. (Figure 1)

In general it was noted that resistance to β-lactam with a low 33% rates. Even the association of pinicilline novobiocin (PNV) has not shown efficacy against the tested strains (Figure 2).

It is lower for aminoglycosides with a tau of 18% whose sensitivity profile of the various tested aminoglycosides is variable. With the exception of amikacin that is the most effective (Figure 3).

Figure 4showed some photos of the antibiogram of some strains tested.

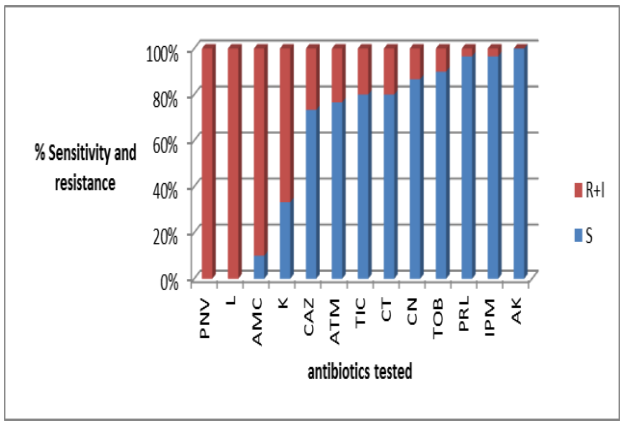

Figure 1: Results of the strains tested susceptibility patterns to antibiotics.

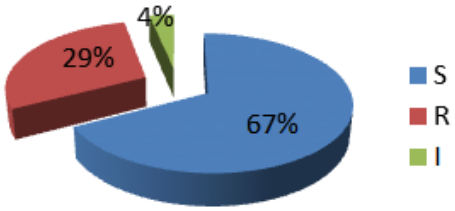

Figure 2: Results of the susceptibility patterns beta

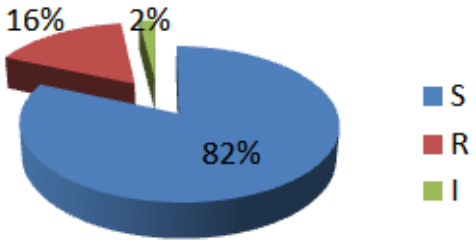

Figure 3: Results profiles sensitivity to aminoglycosides

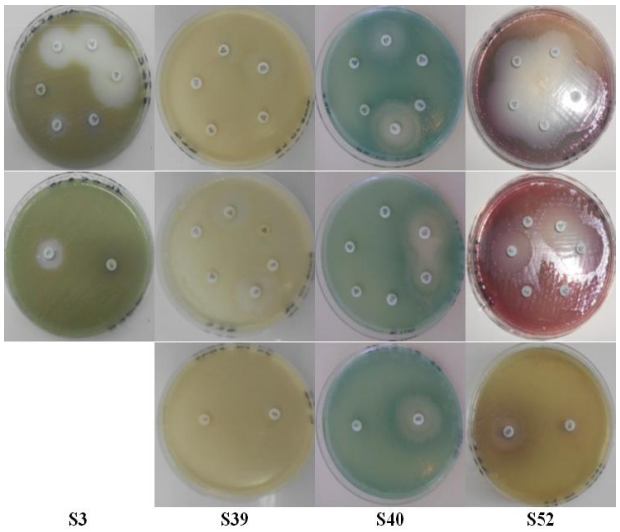

Figure 4: Some photos of the antibiogram of some strains tested

Antiseptogram

The results of the antiseptogram (Figure 5) have shown that hydrogen peroxide at 10 volume is the most effective antiseptic on the *P. aeruginosa* strains tested. The zones of inhibition are very important (25mm to 45mm) relative to alcohol iodinated to 10%. This gave inhibition zones between 7 to 20mm. The surgical alcohol at 70° has resulted very weak inhibition zones (6 à15mm).

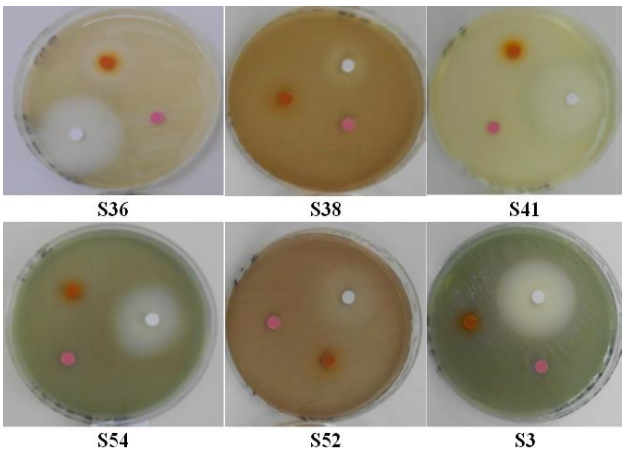

Figure 5: Some photos of anti septogramme of some strains tested

Search inhibitory concentrations of chlorinated water

The research results of inhibitory concentrations of chlorinated water at 13 ° Chl, show that the tested strains can tolerate concentrations of chlorinated water to 0.5%.Only 20% of the strains tested were inhibited by this concentration. From concentration of 1%, chlorinated water showed an inhibitory activity for the strains tested (Figure6. Plate 3)

For confirmation of the inhibition, seeding negative tubes (show no disorder) on solid medium has shown that all strains are tolerant to the concentration of 0.5% in chlorinated water; 13.3% of the strains remain viable even at the concentration of 1% and 6.6% stem bore the concentration of 2% (Figure 7, 8). Therefore, the strains tested show some resistance to chlorination, wherein the concentration of chlorinated water at 2% can only have a bacteriostatic effect on the tested strains.

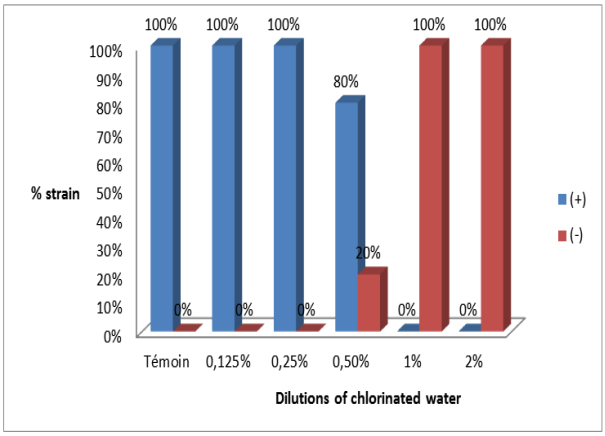

Figure 6: Results of the strains tested susceptibility patterns to different concentrations of chlorinated water

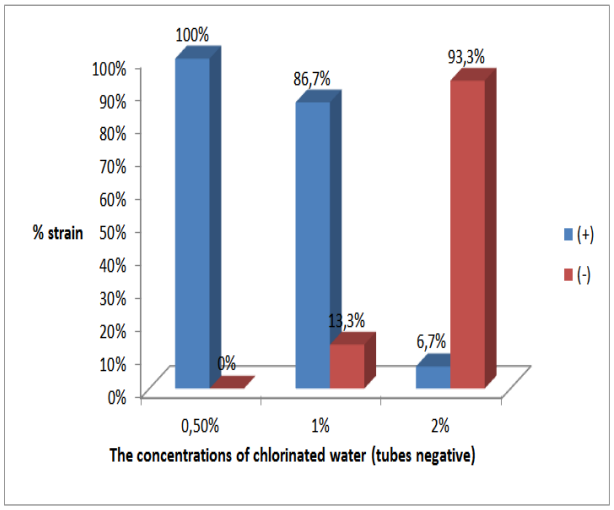

Figure 7: Results of confirmation of inhibition by chlorinated water on solid medium

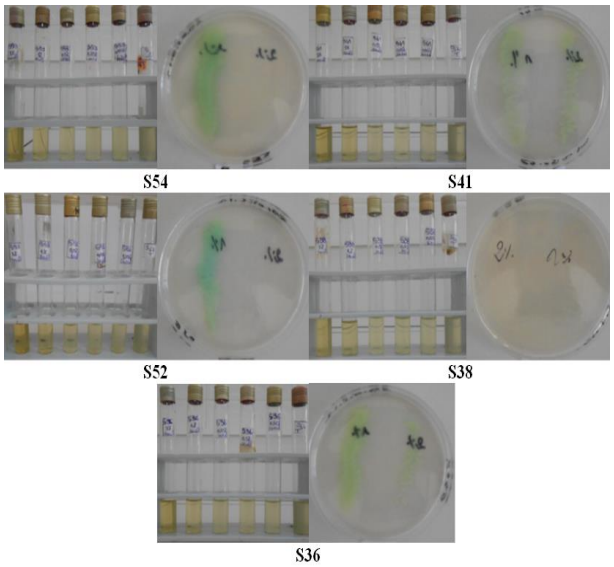

Figure 8: Some photo of the results of stem susceptibility profiles tested with chlorinated water

Amino glycoside in particular the amikacin is considered in literature as effective. Even the strains tested in this work have shown a remarkable sensitivity to this antibiotic as compared to other antibiotics tested.

Our study showed that only 13.3% of the tested strains were resistant to this antibiotic. Therefore gentamicin may be a drug of choice for some ill when the need to resort to the same antibiotic susceptibility in some countries if the use of this antibiotic is less secure than before.

This study allowed us to observe a significant increase in resistance to ceftazidime with a difference of 17.6%. While it did not reveal an evolution of resistance to imipenem which presented the lowest resistance rates for these three periods. Similarly the piperacillin and amikacin were found still effective against the strains tested.

Carmeli and al[23]have studied the risk of emergence of resistance associated with piperacillin, ceftazidime, ciprofloxacin and imipenem; they have observed a maximum risk for imipenem.

Results of Mohnarin showed that *P. aeruginosa* ranked second among all bacteria seen in nosocomial infections. Amikacin, levofloxacin, carbapenem, ceftazidime, and cefepime are usually employed to treat *P. aeruginosa* infections, but its resistance to these drugs and even multi-drug resistant *P. aeruginosa* is already rising year by year. Results of Mohnarin showed that the resistant rates of *P. aeruginosa* to the above-mentioned antibacterial agents were 21.9%, 31.7%, 33.2%, 29.9%, and 25.3%, respectively[24]. In addition, the US MYSTIC surveillance results were 10.4%, 22.4%, 7.3%, 9.8%, and 4.8%, respectively[25-27].

The Korean National Survey of Antimicrobial Resistance has reported that 21%, 22%, and 22% of *P. aeruginosa* strains were resistant to ceftazidime, cefepime and imipenem, respectively[28].

Similar results were seen in a report from 12 tertiary hospitals in Korea, in which 21%, 22%, and 26% of *P. aeruginosa* strains were resistant to ceftazidime, cefepime and imipenem, respectively[29]

Howerver, Sun and alfound that 3.8%, 20.4%, and 2.4% of the *P. aeruginosa* strains isolated from patients were resistant to ceftazidime, ceftazidime, and imipenem, respectively. Differences between results may be due to differences in treatment modality and the frequency of antibiotic used in each diseases[6].

The resistance of *P. aeruginosa* to beta-lactams is due to the existence of metallo-beta-lactamases and extended spectrum beta-lactamases, which confers resistance to most beta-lactam antibiotics, [30] *pseudomonas* are bacteria that accumulate many antibiotic resistance mechanisms, imposing a formal request for confirmation of the effectiveness of selected antibiotics[31,32].

Indeed, *pseudomonas* possesses genetic factors likely to gain or lose the genes of antibiotic resistance, described as the integrons[33, 34]. These integrate can accommodate cassettes, mobile elements formed of a gene and a specific recombination website[35,36].

Several classes integrons have been defined; three of them (classes 1, 2 and 3), well characterized, are involved in the spread of antibiotic resistance[37]

The mechanism of the multi-drug resistance of *P. aeruginosa* mainly lies in the activation of bacterial active efflux systems and the loss of outer membrane protein. The major active efflux systems include MexAB-OprM, MexCD-OprJ, and MexEF-OprN, which can be activated by the induction of some antibacterial agents or chemical agents such as meropenem, quinolones, and detergents[38].

Incorrectly prescribed antibiotics also contribute to the promotion of resistant bacteria. Studies of Centers for Disease Control and Prevention have shown that treatment indication, choice of agent, or duration of antibiotic therapy is incorrect in 30% to 50% of cases[39].

Some strains tested showed resistance to even the most antiseptic used with a tolerance at concentrations of the order of 2% by chlorinated water at 13 °.

Thus, this bacterium could escape inhibition by all antibacterial molecules. This probability calls to focus on the means of prevention to limit infections by *Pseudomonas* against which antibiotics is difficult.

The risk management phenomenon of movement and spread of antibiotic resistance requires the existence and coordination of monitoring networks, to account for the colossal work to have an overall picture taking into account all possible routes of transmission of resistance, common sense recommends action at the root of the problem.

Does the fight against microbial resistance needs reduction and rational use of antibiotics in both human and veterinary medicine which means a sale monitoring and antibiotic consumption? And is it possible to apply it outside the hospital?

Information of health professionals, farmers, food producers, industrial and consumer shows essential to make them aware of the problem where everyone is an actor at his level, and to encourage all means of prevention transmission resistance.

Furthermore does the emergence of resistant strains require the search for new antibiotics?

The improved prognosis of infections due to *P. aeruginosa* depends on how early the intensity and effectiveness of antibiotic therapy instituted probabilistically (empirical) initially and then possibly modified according to data from antibiogram and the clinical course. Must carry out bacteriological samples before and during treatment to the bacteriologist may

make available to the clinician one or more therapeutic alternatives to minimize the likelihood of treatment failure. But this practice is not present in medical practice.

Very specific therapeutic adjuvants such as inhibitors of efflux pumps that allow decreases in doses or sensitivities restoration are under development.

But before all, prevention is the key to the problems posed by *P. aeruginosa*. It goes through the strict application by all rules of hygiene, rational use of antibiotics and the use of laboratory to base treatment on susceptibility testing data. But why not think about the practice of using disinfectants?

CONCLUSION

To contribute to the monitoring of multi resistance *Pseubomonas aeruginosa*, the results of the research profiles of sensitivities of strains collected against amino glycosides and beta-lactam antibiotics most used in Algeria showed that the tested strains have high resistance rates (60% to 100%) to penicillin, the novobiocin, the lincomicine, amoxicillin-acideclavulanique, and kanamycin. Antibiotics that are effective on the strains tested are: Amikacin (AK), with an 0% resistance tau and imipenem (IPM) and piperacillin (PRL) with a too low rate of resistance (3.3%).

The prevalence of resistance to imipenem was 81.53%. What to conclude that the evolution of resistance was not important to amikacin, and piperacillin imipinème. Even these antibiotics are always active, and show that they are the most effective antibiotics against *Pseudomonas aeruginosa* Antimicrobial susceptibility remains an indispensable application before prescribing the most effective antibiotic to prevent treatment failures.

The antiseptogram showed that hydrogen peroxide (at10 volume) is most effective on the strains tested with respect to the surgical alcohol 70 ° and iodinated alcohol at 10%.

The research of the inhibitory concentration of chlorinated water at 13 ° on the tested strains shows that these strains can grow in the presence of concentrations of 0.25 to 1% of this disinfectant. In addition there are some which can withstand more than 2% concentrations. This could explain the causes of the increase in nosocomial infections with *Pseudomonas aeruginosa*, and especially those postoperative. So Systematic surveillance of antibacterial resistance coupled with medical education will allow a more rational use of antibiotics and help control increases in bacterial resistance.

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