

Antimicrobial Drug Resistance and Disinfectants Susceptibility of *Pseudomonas aeruginosa* Isolates from Clinical and Environmental Samples in Jimma University Specialized Hospital, Southwest Ethiopia

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Abstract: *P. aeruginosa* is one of the most important pathogen that causes nosocomial infections often acquired from hospital environment and contaminated medical devices. The infections caused by this bacterium are particularly problematic because it is inherently resistant to several unrelated antimicrobial agents and antiseptics. Hence, the aim of this study was to determine drug resistance and disinfectants susceptibility of *P. aeruginosa* isolated from clinical samples and hospital environments. A laboratory based cross sectional study was conducted from May to September, 2012 on a total of 81 *P. aeruginosa* isolates. A standard bacteriological technique (conventional biochemical tests and pigment production) was used to identify the bacterium. Drug resistance and disinfectant susceptibility tests were determined by Kirby-Bauer disc-diffusion and through the classic method of successive dilutions respectively. In this study a total of 305 (160 clinical and 145 environmental) samples were investigated for *P. aeruginosa* and 81 isolates were obtained. This gives an overall *P. aeruginosa* isolation rate of 26.5% (81/305). About 47.5% of the clinical and 34.2% environmental isolates were detected from wounds and ward sinks respectively. Of these *P. aeruginosa* isolates, 95.1% were resistance to Trimethoprim-sulphamethoxazole, 62% to Gentamicin, and 58% to Ceftriaxone. But, only 4.9% of isolates were resistance to Amikacin. Moreover, disinfectant susceptibility test revealed that hydrogen peroxide and sodium hypochlorite had a higher (92.6% & 91.4%) bactericidal activity compared to ethanol and savlon at the recommended user dilution. In general, our results indicated that *P. aeruginosa* was significantly resistance to commonly prescribed antimicrobial drugs, a situation that demands a more rational and appropriate use of antibiotics. Hydrogen peroxide and sodium hypochlorite were relatively more effective when used in recommended dilution. However, all tested disinfectants or antiseptics showed reduced bactericidal activities in higher dilutions. Therefore, strict adherence to the recommended dilution is important for better activity. The correct use of them also has to be considered appropriately as part of infection control practices.

Keywords: *P. aeruginosa*, Drug resistance, Disinfectant susceptibility, Clinical and Environmental samples, Jimma

1. Introduction

Pseudomonas aeruginosa belongs to a vast genus of aerobic, non-fermenting, saprophytic, Gram-negative bacilli widespread in nature, particularly in moist environments [1]. This bacterium is primarily a nosocomial pathogen responsible for 10-15% of nosocomial infections and 65% of mortality in hospitals all over the world [2]. Although it causes disease in healthy individuals, it is a

major threat to hospitalized and immunocompromised patients, particularly those with diseases such as burns and cancer. The high mortality associated with these infections is due to a combination of weak host defense system and bacterial resistance to antibiotics [3]. Apart from the patient's predisposing factors, the risk of *P. aeruginosa* infection can be greatly increased by inadequate hospital

hygiene. The hospital environment and contaminated medical devices have often been suggested as potential sources of infection [4]. As such, contaminated respiratory care equipment, irrigating solutions, catheters, infusions, dilute antiseptics, cleaning liquids, and even soaps have been reported as vehicles of transmission [1].

Naturally, this organism is endowed with weak pathogenic potentials. However, its profound ability to survive on inert materials, minimal nutritional requirement, tolerance to a wide variety of physical conditions and its relative resistance to several unrelated antimicrobial agents and antiseptics, contributes enormously to its ecological success and its role as an effective opportunistic pathogen [1]. Since the majority of *P. aeruginosa* strains are resistant to most of antibacterial agents, it is considered as one of the major problems in many hospitals [5]. These resistant strains establish themselves in the hospital environment in areas like sinks, taps, railing, mattress, toilets, and thereby spread from one patient to another. Its high rate for developing resistance against most of the antimicrobial agents have caused attention to be focused on measures for fighting resistance, foremost of which is susceptibility surveillance [6].

Antiseptics and disinfectants are used extensively in hospitals and healthcare settings for a variety of topical and hard surface applications. In particular, they are an essential part of infection control practices and aid in the prevention of nosocomial infections in general [7]. However, infections by *P. aeruginosa* are often difficult to treat because of its virulence, intrinsic and acquired antibiotic resistance, and limited choice for effective antimicrobial agents. It is also particularly resistant to biocides (disinfectants, antiseptics, and preservatives). Antibiotic resistance in bacteria has been subject of a great deal of research. By contrast biocide resistance is an emerging issue that is now attracting interest [8, 9]. The selection, use and control of the effectiveness of disinfectants have been emphasized, since environmental surfaces and medical and surgical instruments can serve as vehicles for infectious agents in susceptible hosts associated with the hospital setting [7].

Although different strategies are designed for the control of antimicrobial drugs and disinfectants resistance, most countries to date have focused on two main strategies: (i) the appropriate and prudent use of antimicrobial drugs and disinfectants both in hospitals and the community and (ii) good infection control practices again both in hospitals and the community [10]. Moreover, effective means to control antimicrobial drugs resistance is to develop a surveillance programme on a national and international level. This would be of great assistance, especially for forecasting future changes in the resistance of bacteria [11]. Therefore, this study aimed to assess pattern of drug resistance and disinfectant susceptibility of *P. aeruginosa* isolated from clinical and environmental samples. The findings of this study would indicate selection of proper antimicrobial agent for hospital use and better management of hospital infections.

2. Materials and Methods

2.1. Study Design and Population

A hospital based cross sectional study was conducted to assess the antimicrobial drugs and disinfectants susceptibility pattern of *P. aeruginosa* isolated from clinical and environmental samples. The study was undertaken from May 1 to September 30, 2012 in Jimma University Specialized Hospital (JUSH). It is the only referral hospital for over ten million people in the southwestern part of the country. The hospital provides almost all major types of medical care in its different departments. It is also a teaching hospital with a total capacity of about four hundred beds and eleven wards [12].

2.2. Specimen Collection and Processing

Clinical specimens such as urine, sputum and swabs from wounds/burns were collected from hospitalized and non-hospitalized patients attending in JUSH for various medical reasons. Environmental specimens from patients' beds, table tops, benches tops, door handles, and ward sinks were collected by rubbing moist sterile cotton swabs on these material surfaces. Then, the swabs were placed into test tubes having 0.5 ml of normal saline solution and transported to the laboratory within 30 min to 1 hr of collection [13].

Subsequently, both clinical and environmental specimens were inoculated on MacConkey agar (Oxoid, England) and incubated at 37°C for 18-24 hours. Identification of *P. aeruginosa* was done using colony morphology, Gram-stain and conventional biochemical tests such as oxidase production, citrate utilization, and oxidative fermentation. Pigment production was confirmed by sub-culturing suspected colonies on nutrient agar (Oxoid, England) [13].

2.3. Antimicrobial Drugs Susceptibility Testing

The drug susceptibility testing of *P. aeruginosa* isolates was done by disc diffusion method following Clinical Laboratory Standards Institute (CLSI) guide lines [14]. Accordingly, 3-5 selected colonies of *P. aeruginosa* were taken from a pure culture and transferred to a tube containing 5 ml sterile nutrient broth and mixed gently until a homogenous suspension was formed. The test organisms (bacterial isolates) was grown in nutrient broth and incubated for 4-6 hours at 37°C until the turbidity was matched with the 0.5 McFarland standards. The bacteria suspension adjusted to McFarland turbidity was evenly swabbed over the entire surface of Mueller Hinton agar (Oxoid, England) using sterile cotton swab. The inoculated plates were then incubated at 37°C for 24 hours. Diameters of the zone of inhibition around the discs were measured to the nearest millimeter using a ruler, which was held on the back of the inverted petri plate, and the isolates were classified as sensitive, intermediate and resistant according to the standardized table supplied by the CLSI [14].

The drug discs were obtained from Oxoid, England, with the following concentrations: Amikacin (AN) (30µg), Ceftriaxone (CRO) (30µg), Gentamicin (CN) (10µg), Norfloxacin (NOR) (10µg), Ciprofloxacin (CIP) (5µg), Piperacillin (PIP) (100µg), Imipenem (IPM) (10µg), and Trimethoprim-sulphamethoxazole (SXT) (1.25/23.75µg). The criterion used to select the antimicrobial drugs was based on guideline provided by CLSI for the management of pseudomonas infections, their availability and from literatures search. The standard reference strain of *P. aeruginosa* (ATCC 27853) was used as a quality control for culture and susceptibility testing throughout the study.

2.4. Disinfectants Susceptibility Testing

There are several methods of testing susceptibility of a test organism to a given disinfectants/antiseptics or to ascertain potency and efficacy of a particular chemical antimicrobials. In this study the susceptibility of *P.aeruginosa* isolates were tested against Hydrogen peroxide (3% w/v), Sodium hypochlorite (5% w/v), Ethanol (70% v/v) and Savlon (1.5% v/v Chlorhexidine + 15% w/v Cetrimide). The bactericidal concentration of these four commercial disinfectants and/or antiseptics was determined through the classic method of successive dilutions. In series of seven test tubes (which labeled as # 1-7), 1 ml of sterile nutrient broth was distributed into every tube, except tube # 1. Then, 1 ml of disinfectant of known concentration was added into the 1st and the 2nd tubes of the series. The content in tube 2 was mixed and 1 ml mix was transferred into tube 3. This successive transference was repeated until tube 5 and at last 1ml of content from tube 5 was discarded into sink. Finally, 0.1 ml of *P.aeruginosa* suspension was added to all tubes, except tube # 7. Tube #6 was used as positive control with its content (nutrient broth + test organism) and Tube #7 as negative control (nutrient broth + distilled water). The contents of the tubes were then incubated at 35-37°C for 18-24 hours. At last, sub-culturing the contents of the tubes was made on nutrient agar and observed for bacterial growth after 24 hour of incubation at 35-37°C. The bactericidal concentration was considered as the concentration of the tube in which no *P. aeruginosa* growth was seen after sub-culturing the contents of the tube on nutrient agar [15]. As a quality control, the standard reference strain of *P. aeruginosa* (ATCC 27853) was used in order to check quality of nutrient broth and all disinfectants were kept in the dark at room temperature and freshly prepared prior to testing.

3. Data Analysis

The relevant data was collectively documented on a questionnaire and analyzed using SPSS version 16.0.

4. Results

A total of 305 (160 clinical and 145 environmental)

samples were investigated for *P. aeruginosa*. Correspondingly, 81 isolates (40 from clinical and 41 from environmental samples) were obtained with an overall isolation rate of 26.5% (81/305). Of those clinical isolates, 47.5% was identified from wounds, 37.5% from urine and 15% from sputum samples (Table 1).

Table 1. Frequency of *P. aeruginosa* isolates with respect to clinical specimen types at Jimma University Specialized Hospital, Jimma, Southwest Ethiopia (May-September 2012).

Specimen	Specimens examined	<i>P. aeruginosa</i> isolates; No. (%)
Wound	55	19(47.5)
Urine	55	15(37.5)
Sputum	50	6(15)
Total	160	40(100)

Of those various environmental samples, the highest rate (34.2%) of *P. aeruginosa* was detected in ward sinks, followed by door handles (26.8%) and beds (19.5%) (Table 2).

Table 2. Frequency of *P. aeruginosa* isolates with respect to housekeeping surfaces at Jimma University Specialized Hospital, Jimma, Southwest Ethiopia (May-September 2012).

Site of collection	Samples examined	<i>P. aeruginosa</i> isolates; No. (%)
Ward sinks	30	14(34.2)
Door handles	29	11(26.8)
Patients' beds	29	8(19.5)
Table tops	29	5(12.2)
Bench tops	28	3(7.3)
Total	145	41(100)

The drug susceptibility testing profile of *P. aeruginosa* showed that, 95.1% of isolates were resistance to Trimethoprim-sulphamethoxazole, 62% to Gentamicin, and 58% to Ceftriaxone. But, only 4.9% of isolates were resistance to Amikacin. The *P.aeruginosa* isolates from environments were more resistant to a particular drug than that of clinical isolates. However, the observed difference was not statistically significant ($P>0.05$) (Table 3).

Table 3. Antimicrobial drugs resistance of *P.aeruginosa* (n=81) with respect to sample types at Jimma University Specialized Hospital, Jimma, Southwest Ethiopia (May-September 2012).

Drugs	Sample types		Total No(%)	P-value
	Clinical isolates No(%) resistance	Environmental isolates No(%) resistance		
Amikacin	1(2.5)	3(7.3)	4(4.9)	0.360
Gentamicin	22(55)	28(68.3)	50(62)	0.221
Imipenem	3(7.5)	7(17.1)	10(12.3)	0.245
Ceftriaxone	21(52.5)	26(63.4)	47(58)	0.591
Piperacillin	2(5)	7(17.1)	9(11.1)	0.224
Ciprofloxacin	5(12.5)	12(29.3)	17(21)	0.135
Norfloxacin	3(7.5)	9(21.9)	12(14.8)	0.145
Trimethoprim-sulphamethoxazole	39(97.5)	38(92.7)	77(95.1)	0.368

Moreover, the disinfectant susceptibility testing in this study confirmed that at user dilution (0.5% concentration)

of sodium hypochlorite showed lethal effect on 91.4% of the *P. aeruginosa* isolates. Similarly, Hydrogen peroxide at user dilution (3% concentration) was found to be effective against 92.6% of isolates. At its recommended dilutions, 2% Savlon® and 70% ethanol exhibited bactericidal effect against 88.9% and 85.2% of *P. aeruginosa* isolates respectively (Table 4).

Table 4. Disinfectants susceptibility of *P. aeruginosa* (n=81) isolates from clinical and environmental samples in Jimma University Specialized Hospital, Jimma, Southwest Ethiopia (May-September 2012).

Dilutions	No (%) of isolates susceptible to Disinfectants/antiseptics type			
	Hydrogen peroxide	Sodium hypochlorite	Ethanol	Savlon®
User (Recommended)*	75(92.6)	74(91.4)	69(85.2)	72(88.9)
1:2	70(86.4)	65(80.2)	42(51.8)	57(70.4)
1:4	44(54.3)	61(75.3)	0(0)	35(43.2)
1:8	21(25.9)	57(70.4)	0(0)	12(14.8)
1:16	5(6.2)	52(64.2)	0(0)	1(1.2)
1:32	0(0)	40(49.4)	0(0)	0(0)

*User dilution: Hydrogen peroxide-3%; Sodium hypochlorite- 0.5%; Ethanol-70%; Savlon®-2%

5. Discussion

In this study, *P. aeruginosa* was identified from all four specimen types (urine, wound, sputum and environmental swabs) with an overall isolation rate of 26.5% (81/305) reflecting the ubiquitous nature of the organism. This finding goes in agreement with the study in Cameroon where 25.5% detection rate was reported [1] but higher than 18.6% reported in Egypt [16]. In our study, the 25% detection rate of *P. aeruginosa* in clinical specimens was not significantly different from that of 28.3% from environmental specimens ($P > 0.05$). Similar results were reported in Cameroon [1] and Egypt [16]. Majority (90%) of clinical *P. aeruginosa* isolates in present study were identified on samples taken from admitted patients. Such frequent isolation of *P. aeruginosa* from hospitalized patients substantiated its significant role in nosocomial infections.

In this study, the majority (34.2%) of *P. aeruginosa* identified in the environmental samples were from ward sinks, which could be explained by the fact that the bacterium thrives very well at habitats with adequate amount of moisture. Door handle samples gave the next highest proportion (26.8%) of isolates. In hospitals and other places of human habitation, door handles are the most common article of contact by the people. It is therefore not surprising thriving of high *P. aeruginosa* isolates in such sites since people with wet hands (water or sweat) may easily come into contact with it. The places with least number of isolates were the beds, tables and benches tops as these are places that are likely to be dry.

Resistance of *P. aeruginosa* to commonly used antimicrobial agents is becoming an increasing clinical problem and a recognized public health threat [3]. In the

present study, the highest resistance rate of *P. aeruginosa* was observed for Trimethoprim-sulphamethoxazole which was 97.5% and 92.7% in clinical and environmental isolates respectively ($p > 0.05$). This finding is in agreement with 87.5% reported in Addis Ababa [17], 93.5% in Bangladesh [18], 100% in Iran [6] and Nigeria [19] each. The possible explanation for such high rate of resistance against Trimethoprim-sulphamethoxazole might be associated with its easily availability and indiscriminate usage.

The resistance rate of *P. aeruginosa* isolates in clinical and environmental isolates to Gentamicin was 55% and 68.3% respectively ($p > 0.05$), with an overall resistance of 62%. This finding goes in line with studies done in India (63%) [20], Cameroon (66.7%) [1], Egypt (67.5%) [16] and Turkey (57.5%) [21], but higher than reports from Jamaica (21.6%) [22], Ghana (46%) [23], Nigeria (53%) [24] and Iran (52.2%) [25]. Higher resistance rates were also reported from Jordan (72%) [26], Bangladesh (77.3%) [18], Saudi Arabia (85.3%) [27] and Malaysia (94.3%) [28]. Bacterial resistance to Gentamicin is mainly due to an enzymatic modification of the antibiotic as it was indicated by Poole K [29]. The widely use of this drug in the hospital setting may have also contributed for the high resistance rate seen in this study.

The rate (58%) of resistance of the *P. aeruginosa* isolates to Ceftriaxone seen in this study is in agreement with documented in Addis Ababa (58.3%) [17], Iran (58.3%) [30], Egypt (63.3%) [16] and Nigeria (65%) [24]. However, relatively higher resistance rates were reported from Palestine (75%) [31], Turkey (84.2%) [21], Bangladesh (86.1%) [18] and Nigeria (86.8%) [19]. The high level of resistance seen against Ceftriaxone may be due to difference in intense and indiscriminate usage of the drug in these countries.

Although *P. aeruginosa* resistance to antibiotics has been extensively studied in both clinical and environmental samples, only few reports were available on disinfectants/antiseptics activity against this bacterium. It is well known that Sodium hypochlorite is widely used for disinfection of environmental surfaces, including surfaces contaminated by blood spills [32]. In the present study, evaluation of the susceptibility of *P. aeruginosa* to the sodium hypochlorite disinfectant demonstrated that the recommended user dilution (with 0.5% free chlorine) was effective against 91.4% of the isolates. This finding showed that sodium hypochlorite stands as an effective disinfectant formulation against *P. aeruginosa* when used at the recommended concentration. This finding goes in line with study reports made in USA [33] and Brazil [34] where sodium hypochlorite, at a concentration of 0.5%, was effective against 92.2% and 86% of *P. aeruginosa* isolates respectively.

Hydrogen peroxide (H_2O_2) is a widely used biocide for disinfection and antisepsis [33]. In the current study, the chemical has showed bactericidal activity against 92.6% of *P. aeruginosa* isolates at the user dilution of 3% concentration. This study also demonstrated that about 86.4% of tested

isolates were susceptible at 1:2 dilutions (1.5% H₂O₂) too.

Alcohols exhibit rapid broad-spectrum antimicrobial activity against vegetative bacteria, viruses, and fungi but bacterial spores. It is widely used for both hard-surface disinfection and skin antisepsis. Little is known about the specific mode of action of alcohols, but based on the increased efficacy in the presence of water; it is generally believed that they cause membrane damage and rapid denaturation of proteins, with subsequent interference with metabolism and cell lysis [33]. In this study a total of 85.2% *P. aeruginosa* isolates were killed at the recommended 70% concentration. This finding shows that resistance is developing for 70% ethanol, although it remains the disinfectant of choice in hospitals.

Savlon® works well on minor cuts and abrasions, promoting fast healing and minimal scarring. In the present study, Savlon® exhibited bactericidal effect against 88.9% of *P. aeruginosa* isolates at the recommended dilution (2% concentration). The 1:2 dilutions (1% concentration) of it found to be effective against 70.4% of the isolates. Comparable finding was reported from study done in Nigeria where 61% of *P. aeruginosa* isolates were susceptible to the Savlon at 1% concentration [35].

As indicated by McDonnell G. and Russell AD, reduced susceptibility of *P. aeruginosa* to any disinfectants can be associated with the ability of the bacterium to form biofilms. Growth within biofilms gives rise to extensive genetic diversity that, in turn, enhances the potential for resistance against disinfectants, which can be attributed to reduced access of antiseptic or disinfectant to underlying cell, modulation of the microenvironment and genetic exchange between cells in a biofilm, which enhances tolerance to antiseptics and disinfectant [33].

6. Conclusion

Our results indicated that *P. aeruginosa* was less susceptible to commonly prescribed antimicrobial drugs which is an indication of circulating high levels of drug resistance strains, a situation that demands immediate attention. In spite of this, Amikacin, Piperacillin and Imipenem showed very good activity so that these antibiotics seem to be a promising therapy for pseudomonas infection. Since, empirical treatments to pseudomonas infections provoke drug resistance; treatment is advised to be based on the result of culture and sensitivity.

In this study, sodium hypochlorite, hydrogen peroxide, Savlon® and ethanol were relatively most effective when used in recommended dilution. However, all tested disinfectants or antiseptics showed reduced bactericidal activities in higher dilutions. The impaired effectiveness of disinfectants is worrisome as they are critical components of intervention strategies used in clinical medicine for preventing the dissemination of nosocomial diseases and their applicability in community environments for personal hygiene. The reduced susceptibility probably occurs as a result of indiscriminate and constant use and misuse of a

particular disinfectant for a long period of time. Therefore, strict adherence to the recommended dilution of these chemical agents is compulsory for better activity, and the correct use of them also has to be considered appropriately as part of infection control practices.

7. Ethics

Official permission was obtained from the Jimma University Specialized Hospital management committee for doing the study in the hospital and written informed consent was also obtained from patients prior to sampling. All laboratory tests were done free of charge and their results were communicated to patients respective physician or nurses for beneficiary measures and any information was kept confidential.

Competing interests

The authors declare that they have no competing interests.

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