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Research Article

Identification and Antimicrobial Susceptibility of *Pseudomonas* Spp. Isolated from Neonatal Intensive Care Unit at Misurata Central Hospital, Libya

Eltaweel Mohamed Abdallah[1]*, Al Mahgob Nuria Abdalla[2] and Elgubbi Huda[1]

[1]*Department of Microbiology, Faculty of Science, Misurata University, Misurata, Libya*

[2]*Diabetic Center, Misurata, Libya*

***Corresponding Author:** Eltaweel Mohamed Abdallah, Department of Microbiology, Faculty of Science, Misurata University, Misurata, Libya.

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Abstract

Pseudomonas spp. are Gram-negative bacteria commonly found in soil and water. It is an opportunistic pathogen and an important cause of healthcare-associated infections, particularly among infants in neonatal intensive care units (NICUs). Several reports regarding the outbreaks of *Pseudomonas* spp in NICUs have been reported. Low birth weight pre-term infants were at greater risk of mortality from *Pseudomonas* spp infection than older infants. In this study, fourteen clinical *Pseudomonas* spp. isolates were isolated from different sites at neonatal intensive care unit at Misurata central hospital. Using API 20E system, the isolates were identified as following: *Pseudomonas aeruginosa*, 6; *Pseudomonas cepacia*, 3; *Pseudomonas pseudomallei*, 2; *Pseudomonas fluorescens*, 2; *Pseudomonas luteola*, 1. Tetracycline was found the most effective antibiotic among those used in this study.

Keywords: *Pseudomonas; Neonates; NICU; Outbreak*

Introduction

Nosocomial infection is a major underestimated problem in developing countries leading to high neonatal mortality rate [1]. Poor hand-hygiene practices [2,3], re-use of single-use medication vials and devices [4], and inadequate sterilization of medical equipment [5] are key proximate events that facilitate transmission of nosocomial pathogens. Moreover, poor management and limited resource to fund infection-control programs as well as overuse of empirical antibiotic therapy were found contributing factors to nosocomial pathogens transmission [6]. *Pseudomonas* spp are known to colonize different hospital environments, particularly moist areas like tap of water, sink, respiratory equipment, bronchoscope, and some antiseptic solutions. Moreover, healthcare workers are a possible reservoir contributed in nosocomial outbreaks [7]. Due to the ubiquitous nature of *P. aeruginosa* causes nosocomial infections as a result of its ubiquitous nature, ability to survive in moist environments, and resistance to many antibiotics and antiseptics [8]. *Pseudomonas cepacia* organism has been identified to be capable of degrading more than 100 different organic molecules. This capability is often leading to cause contamination of equipment, drugs and also in disinfectant solutions in the hospitals thus evolving the organism as a notorious nosocomial pathogen [9]. *P. pseudomallei* has a saprophytic nature and capable of surviving in a relatively hostile environment, but also demonstrates significant surviving capabilities during its interaction with the host immune system [10]. The main reservoir for *P. pseudomallei* is the contaminated environment [11,12]. *P. fluorescens* has generally been regarded to be of low virulence and an infrequent cause of human infection [13]. However, it has been reported to cause infections such as blood transfusion-related septicemia [14,15], catheter-related bacteremia [13], and peritonitis in peritoneal dialysis patients [16]. The normal habitat of *P. luteola* is unclear, although it belongs to a group of bacteria normally found in moist environments [17], they can contaminate solutions such as distilled water, disinfectants and

intravenous solutions [18]. *P. luteola* may cause bloodstream infections associated with intravenous indwelling catheters, prosthetic valve endocarditis, pancreatitis, foreign bodies and cutaneous abscesses [19]. The ubiquitous nature of *P. aeruginosa* in the environment makes the sources of such outbreaks difficult to identify. The aim of this study was to determine the rate of existence and antibiotic susceptibility of *Pseudomonas* spp at neonatal intensive care unit in Misurata central hospital.

Materials and Methods

Study sitting and sample collection

Study was conducted in Misurata central hospital, one of the referral hospitals in Misurata, Libya. Isolates were obtained from different sites and medical devices of NICU of the hospital. The sites include baby cabinets, blankets, doors handle, diagnostic tables, nasogastric tube, suction machine, artificial ventilation, and pulse oximeter. The samples were taken with wet sterile cotton swabs. Isolates were identified by following the standard microbiological methods as described by Greenwood., *et al*. [20] and confirmed by oxidase test and API 20E system. The study was conducted from October 2014 to June 2015.

Antimicrobial susceptibility

Using disk diffusion test, *Pseudomonas* isolates susceptibility to the following antimicrobial agents was evaluated: Oxacillin (OX), Chloramphenicol (C), Rifampicin (RD), Sulphamethoxazole/Trimethoprim (SXT), Amoxicillin-clavulanic acid (AMC), Gentamicin (CN), Piperacillin (PRL), Ampicillin (AMP), Ceftriaxone (CRO), Aztreonam (ATM) and Tetracycline (TE).

Selected *Pseudomonas* isolates were cultured in nutrient agar for 24h at 37°C. Pure colony of each selected *Pseudomonas* isolates were inoculated into 10 ml of nutrient broth and their turbidity adjusted to 0.5 McFarland standard. Cotton swab from the prepared broth was spread on Mueller-Hinton agar and incubated for 24h at 37°C. Zone of inhibition were measured and isolates antimicrobial susceptibility evaluated according to standard table [21].

Results and Discussion

A total of 14 *Pseudomonas* spp were isolated at this study. A commercially available bacterial identification test (API 20-E bioMerieux) was used, as conventional biochemical reactions for definitive identification of the *Pseudomonas* isolates. Six of them were identify as *Pseudomonas aeruginosa*, three *Pseudomonas cepacia*, two Pseudomonas pseudomallei two Pseudomonas fluorescens, and one was *Pseudomonas luteola* (Table 1).

Sites	Bacterial species									
	P. aeruginosa		*P. cepacia*		*P. pseudomallei*		*P. fluorescens*		*P. luteola*	
	Isolates	%	Isolates	%	Isolates	%	Isolates	%	Isolates	%
Door handles	2	33.33	1	33.3	1	50	1	50	1	100
Diagnostic tables	2	33.33	1	33.3	0	0	1	50	0	0
Nasogastric tube	1	16.67	1	33.3	1	50	0	0	0	0
Suction machine	1	16.67	0		0	0	0	0	0	0
Total of Isolates	6	100	3	100	2	100	2	100	1	100

Table 1: *Frequency of occurrence of Pseudomonas isolates in different sites.*

At this study, *Pseudomonas aeruginosa* showed the highest prevalence among the Pseudomonas spp. isolated from the NICU (42. 86%). The organisms were pecked from the door handles (2 isolates), diagnostic tables (2 isolates), one isolate pecked from nasogastric tube and another one was pecked from the suction machine. Three isolates of *Pseudomonas cepacia* representative 21.43% of the total isolates. One of them was pecked from door handles, another one from the diagnostic tables, and the third one was pecked from nasogastric tube. Two isolates of *Pseudomonas pseudomallei* (14.28%) were found at the NICU, one of these isolates was pecked from the NICU door handles whereas the other one was from the nasogastric tube. *Pseudomonas fluorescens* (14.28%) was pecked from the door handles and diagnostic tables one isolate from each. One isolate (7.15%) of *Pseudomonas luteola* was pecked from door handles.

Study findings showed 6 isolates of *Pseudomonas aeruginosa* pecked from doors handle which was nearly similar to the study done by Ghane and Azimi [21] who reported that seven isolates of *pseudomonas* spp were isolated from doors handle. *Pseudomonas* spp. is known to colonize the hospital environment, particularly moist sites, sources such as tap water, sink, antiseptic solutions, respiratory equipment, and bronchoscopes are the most commonly incriminated nosocomial reservoirs of *Pseudomonas* spp. They also reported that *pseudomonas* spp were also isolated from sinks, water taps, ground flours, medicine cabinet, bed, wall, window, door, nurse table, ashcan surface, chair, electric switch and refrigerator handle and the number of the isolates were 17, 9, 6, 4, 4, 3, 2, 2, 2,2, 1, 1 and 1 respectively, and they mentioned that 61 isolates of *Pseudomonas* spp were isolated from the surfaces in a hospital sections, the most prevalent of *Pseudomonas* spp. found were related to *P. aeruginosa* (52), followed by *P. stutzeri* (6), *P. putida* (2) and *P. fluorescens* (1). Aloma., *et al*. [22] *P. aeruginosa* was isolated from sink (59.4%), floor (34.4%), table tops (6.3%) at the hospital environment.

The result of the antibiotic susceptibility of *Pseudomonas* isolates by disc diffusion method indicated that all strains were resistant (100%) to Oxacillin, Chloramphenicol and Rifampicin, while they showed different levels of resistance to other used antibiotic. Five isolates (35.71%) showed resistance to Sulfamethoxazole /Trimethoprim, Amoxicillin-clavulanic acid, Gentamicin and Piperacillin, eleven (78.57%) to Ampicillin, Ceftriaxone and Aztreonam while they were all sensitive to Tetracycline (Table 2). AL-Marjani., *et al*. [23] reported that all *P. aeruginosa* isolates were resistance to Oxacillin which is in agreement with this study. In contrast, their study showed that they are resistant to Gentamicin and Tetracycline (80 and 100% respectively), and all of *P. aeruginosa* were highly resistant (100%) to Amoxicillin and Tetracycline; whereas the resistance percentages for Sulphamethoxazole/Trimethoprim and Gentamicin were 84% and 80% respectively, which is in disagreement with our study. Farid., *et al*. [24] reported that clinical isolates of *P. luteola* are often resistant to first- and second-generation Cephalosporins and Tetracyclines, Ampicillin, and Sulphamethoxazole /Trimethoprim, but are susceptible to third-generation Cephalosporins, Mezlocillin, Imipenem, Aminoglycosides, and Quinolones. The high frequency of multiple resistance among *P. aeruginosa* isolates makes its control difficult, and mortality associated with *P. aeruginosa* infection is high when compared to other bacteria [25].

Isolates	No. of Isolates	OX	C	RD	SXT	AMC	CN	PRL	AMP	CRO	ATM	TE
P. aeruginosa	6	R	R	R	S	S	S	S	R	R	R	S
P. cepacia	3	R	R	R	S	S	S	S	S	S	S	S
P. pseudomallei	2	R	R	R	R	R	R	R	R	R	R	S
P. fluorescens	2	R	R	R	R	R	R	R	R	R	R	S
P. luteola	1	R	R	R	R	R	R	R	R	R	R	S

Table 2: *Antimicrobial resistance of Pseudomonas isolates by disc diffusion method.*
R: Resistance; S: Sensitive

Pseudomonas cepacia isolates were resistance (100%) to Oxacillin, Chloramphenicol and Rifampicin and sensitive to the rest of tested antibiotic. Alaa [26] and Roy., *et al*. [27] found that chloramphenicol has antimicrobial sensitivity of 86.6% and 60% respectively, on *P. cepacia* isolated from patients with malignancy. Also, Omar., *et al*. [28] reported sensitivity rates of 37.1%. However, disagrees to our finding.

Susan., *et al*. [29] reported that *P. pseudomallei*, showing susceptibility to piperacillin, ampicillin/sulbactam and tetracycline and resistance to Sulphamethoxazole/Trimethoprim which were defer from our finding where is *P. pseudomallei* was resistance to all antibiotic used in this study except Tetracycline. AL-Marjani., *et al.* [23] the result obtained for antibiotic susceptibility of *Pseudomonas* isolates indicated different levels of resistance to antibiotic, among 61 spp. 47 (77%) of them showed resistance to Ampicillin, 37 (61%) to Amoxicillin, 17 (28%) to Ceftriaxone and piperacillin, 3 (5%) to Gentamicin, 41 (67%) to Chloramphenicol, 44 (72%) to Tetracycline. In comparison with the results from our study, there is some agreement in the levels of resistance to antibiotic as the following 35.71% to Amoxicillin-clavulanic acid, Gentamicin and Piperacillin, 78.57% to Ampicillin and Ceftriaxone, in contrast our study showed 100% resistance to Chloramphenicol and 100% sensitivity to Tetracycline. Dorman., *et al*. [30] reported that *P. pseudomallei*, are susceptible to Piperacillin, Ampicillin/Sulbactam, and Tetracycline.

P. fluorescens and *P. luteola* isolates were resistant to all antibiotics used in this study except Tetracycline to which they were sensitive. Hsueh., *et al*. [12] demonstrated that *P. fluorescens* strains isolated from cancer patients showed susceptibility to Gentamicin, and Tetracycline, and resistance to Chloramphenicol, Ampicillin. Farid., *et al*. [24] reported that clinical isolates of *P. luteola* are often resistant to first- and second-generation Cephalosporins and Tetracycline, Ampicillin, and Sulphamethoxazole/Trimethoprim, but are susceptible to third-generation Cephalosporins, Mezlocillin, Imipenem, Aminoglycosides, and Quinolones.

Conclusion

The result of this study showed that inanimate surfaces near patients and those frequently touched surfaces within the hospital environment were contaminated by *Pseudomonas* spp. This suggests that contaminated environmental surfaces are reservoirs of these pathogen. Some of the isolates of the pathogen were multidrug resistant and are common. Therefore, regular surveillance of hospital and community associated *Pseudomonas* spp and their susceptibility to antibiotics are necessary to prevent an outbreak and spread of resistant strains in the locality.

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