Original Research Article

Pseudomonas aeruginosa skin-nasopharyngeal colonization in the In-patients: Prevalence, risk factors and antibiotic resistance in tertiary Hospitals in Sana'a city -Yemen.

Abstract

Pseudomonas aeruginosa is one of most important cause of healthcare-associated infections. This active surveillance cross sectional study was aimed to determine the rate of *P.aeruginosa* colonization among inpatients at three tertiary hospitals in Sana'a city. In addition, to determine risk factors of colonization and the antibiotic susceptibility of the isolated *P.aeruginosa*. The study included 327 inpatients. Skin and nasopharyngeal swabs were collected from all participated patients and data were collected by predesigned questionnaire. Standard methods of isolation and identification were used to isolate bacteria in pure culture then identify. Also, antibiotic sensitivity for isolated *P.aeruginosa* was determined by the disc diffusion method.

42 patients (12.8%) were colonized with *P.aeruginosa* on skin and nasopharyngeal. The significant risk factors of colonization were male patients (OR=2.5), older age (OR= 2.2), burn ward (OR=37), long stay in hospital (OR=4) and burn as underlying disease (OR=45). The isolated *P.aeruginosa* were completely resistant (100%), to Aztroneome, Ceftriaxone, and Ciprofloxacine; and the rates of resistant were ranged between 83.3-85.7% for Amikacin, Ampicillin sulbactam Levofloxacin. Also the rates were 71.4% for Netilmicin and 92.9% for chloramphenicol. Moreover, the rates of resistant were low for Gentamicin (35.7%), Imipenem (11.9%), Pipracillin Tazobactam (11.9%), Ticaracillin calvulanic acid (31%) and Colistin sulphat (14.3%). In conclusion, this study has highlighted the role of hospitalization as a significant risk for *P.aeruginosa* colonization; concerted and coordinated efforts are required both in the hospital and community to tackle this. These data emphasize the need to identify hospitalized patients colonized with *P.aeruginosa* on admission. Prediction rules or rapid diagnostic testing will help clinicians more appropriately choose empiric antibiotic therapy if subsequent infections occurred.

Keywords: active surveillance, antibiotic resistance, colonization, *Pseudomonas aeruginosa*, risk factors, tertiary hospitals

Introduction

Bacterial colonization in general and *P.aeruginosa* colonization in hospitalized patients are important cause of healthcare-associated infections worldwide[1]. In the United States, it is the 6th most common cause of healthcare-associated infections, accounting for 7.1% of all hospital infections.[2] The choice of empiric antibiotics in the hospital setting is difficult. There needs to be a balance between excessively broad coverage and too narrow coverage. Empiric antibiotic coverage that covers *P.aeruginosa* but is broader than necessary may lead to the emergence of *P.aeruginosa* and other intestinal bacteria that are resistant to those broad spectrum antibiotics. In contrast, empiric therapy that does not cover *P.aeruginosa* may lead to poor outcomes for in-patients eventually found to have *P.aeruginosa* infection. Improvements in our understanding of which patients require broad-spectrum empiric coverage versus situations in which narrower spectrum agents may be appropriate would be valuable from an antimicrobial stewardship perspective. Knowledge of whether a patient is colonized with *P.aeruginosa* can be helpful in guiding selection of empiric antibiotics for suspected sepsis in the hospital setting. Colonization with *P.aeruginosa* is associated with subsequent infection with the same strain of *P.aeruginosa* [3, 4]. The objectives of this active surveillance cross sectional study were as follows: a) Determine the prevalence of *P. aeruginosa* colonization on Inpatients; b) Determine risk factors for *P. aeruginosa* colonization; and c) Determine the risk factors of *P. aeruginosa* colonization.

Subjects and Methods

Site of the study

An active surveillance cross sectional study was conducted in in-patients admitted to three tertiary hospitals in Sana'a city: namely: Al-Jumhori hospital, Al-Kuwait hospital and Yemen-Germen hospital, between January 1, 2017, and May 30, 2017. The three hospitals are 1816-bed tertiary care facilities.

Ethical Consideration

Ethical clearance for the study was taken from the Faculty of Medicine and Health Sciences Research Review Committee. A written permission was also taken from the administrative Managers of the Al-Jumhori hospital, Al-Kuwait hospital and Yemen-Germen hospital. Informed Consent was taken from the patients before the questionnaire was filled.

Survey procedure and Laboratory Analysis

Skin and nasopharyngeal swabs collected from inpatients hospitalized in different wards. Clinical and demographic data were collected for all participants' patients. Skin and nasopharyngeal swabs were collected using standard collection techniques, and inoculated on appropriate bacteriological media,

including blood agar and Mac-Conkey. Then plates were incubated aerobically at 37°C for 18–24h. Confirmatory tests include production of the blue-green pigment pyocyanin on Cetrimide agar and growth at 42 °C also done. Also, the identification of isolates was made according to standard methods[5]. The identification was made with basic microbiological methods using colony morphology, Gram staining, oxidase, Indole, catalase and coagulase tests etc [5].

Antimicrobial susceptibility test

Antimicrobial susceptibility test for *P. aeruginosa* isolates were performed on Mueller-Hinton agar plates by the Kirby-Bauer disc diffusion method [5]. The isolates were tested for their susceptibility against 13 antimicrobial agents that are used in Yemen. Standardized suspension of *P. aeruginosa* inoculums was compressed to 0.5 McFarland standard turbidity, then inoculated on 3 Muller-Hinton agar plates using a sterile cotton swab by streaking the swab over the entire sterile agar surface 3 times. The plates then allowed drying and antimicrobial discs were placed at the recommended distance from each other. All plates were aerobically incubated at 37℃ for 24-48 hours before the zones size were record (diameter of inhibition zones was measured and recorded in millimeters with the help of sliding calipers, and an organism was labeled as sensitive or resistance as per CLSI guidelines[5] .

Data analysis

The analysis of data was done by Epi Info version 6 statistical program (CDC, Atlanta, USA), where the chi-square ($\chi 2$) and probability value (p) was calculated for the test of significance. In addition, Odd's ratio (OR), 95% confidence interval (95% CI) were added to estimate the risk factors of skin or nasopharyngeal colonization *P. aeruginosa* on inpatients.

Results

The detailed results of this study are presented in 5 tables. Table 1shows the association between skin, nasopharyngeal *P.aeruginosa* colonization and sex and age of the in-patients. Table 2 demonstrates the association between skin- nasopharyngeal *P.aeruginosa* colonization and type of wards, table 3 illustrates the association between skin- nasopharyngeal *P.aeruginosa* colonization and duration of hospitalization. Table 5 explains the association between skin, nasopharyngeal *P.aeruginosa* colonization and underlying diseases and table 6 shows the antibiotic sensitivity of isolated *P.aeruginosa*.

42 patients (12.8%) were colonized with *P.aeruginosa* on in skin and nasopharyngeal. The significant risk factors of colonization were male patients (OR=2.5,95% CI=1.1-5.5, p=0.01), older age (OR= 2.2, 95% CI= 1.1–4.2, p=0.01), Burn ward (OR=37, 95% CI =16.2-84, p<0.001), long stay in hospital (OR=4, 95% CI =2.0-7.8, p<0.001) and burn as underlying disease (OR=45, 95% CI =19-105, p<0.001). The isolates of *P. aeruginosa* were completely resistant (100%), to Aztroneome, Ceftriaxone, and Ciprofloxacine and the rates of resistant were between 83.3-85.7% for Amikacin, Ampicillin sulbactam Levofloxacin, 71.4% for Netilmicin and 92.9% for chloramphenicol. Moreover, the rates of resistant were low for Gentamicin (35.7%), Imipenem (11.9%), Pipracillin Tazobactam (11.9%), Ticaracillin calvulanic acid (31%) and Colistin sulphat (14.3%).

Discussion

In present study , there was a significant rate of skin- nasopharyngeal *P.aeruginosa* colonization in inpatients equal to 12.8% (table 1). Our result is comparable to that reported from district hospitals in UK [6]. Furthermore our study is similar to that reported previously by Parkins *et al.* in which hospitalization was a risk factor for bacterial colonization [7]. This association can be explained by that high rate of direct transmission of infectious agents in hospitals, in which this involves a direct body surface-to-body surface contact and physical transfer of microorganisms between a susceptible host and an infected or colonized person, such as when a person turns a patient, gives a patient a bath, or performs other patient-care activities that require direct personal contact. Direct-contact transmission also can occur between two patients, with one serving as the source of the infectious microorganisms and the other as a susceptible host[8]; or by indirect-contact transmission, which involves contact of a susceptible host with a contaminated intermediate object, usually inanimate, such as contaminated instruments, needles, or dressings, or contaminated gloves that are not changed between patients. In addition, the improper use of saline flush syringes, vials, and bags has been implicated in disease transmission in the US, even when healthcare workers had access to gloves, disposable needles, intravenous devices, and flushes[8].

Additionally in our study, there was significant association between male patients and skin-nasopharyngeal *P.aeruginosa* colonization in inpatients equal to 2.5 times with CI=1.1- 5.5 and PV=0.01, while no significant association was found with female. Our result is similar to that reported previously by several studies in which male sex was a risk factor associated with contracting bacterial colonization in inpatients [1, 7-12]. As well, there was significant association between older age group ≥50 years and skin-nasopharyngeal *P.aeruginosa* colonization in inpatients equal to 2.2 times with CI=1.1-

4.2 and PV=0.01, while no significant association was found with younger age groups (table 1). This result is parallel to that reported previously by studies in USA and Europe in which older age group was a risk factor associated with contracting bacterial colonization in hospitalized patients [1,10-12].

When we considered the association between skin-nasopharyngeal *P.aeruginosa* colonization and type of wards, there was significant associated OR equal to 37 times with CI=16.2- 84 and PV<0.0001 with burn wards (table 2). This result is similar to that reported by Lyczak, *et al*. in which burn wards were associated risk factor with bacterial colonization in inpatients [13]. This association can be explained by that original thermal injury creates a breach in the surface of the skin which make it more susceptible to colonization with micro-organisms, and *P. aeruginosa* use quorum sensing to induce the production of virulence factors such as proteases, hemolysins, exotoxins A and pyocyanin which help bacteria to colonized [14]. However there was no significant association between ICU wards and skin-nasopharyngeal *P.aeruginosa* colonization (table 2). This result is different from that reported by Harris *et al.* [6] in which significant association between ICU patients and colonized with *P. aeruginosa* after admission to hospitals. Our negative result might be due to our small sample size.

As soon as we considered the association between skin-nasopharyngeal bacterial colonization and duration of hospitalization, there was significant associated OR equal to 4.0 times with CI=2.0- 7.8 and PV<0.001 with ≥17 days of hospitalization (table 3). This result is similar to that reported elsewhere in which longer period of hospitalization was a hazard factor of bacteria colonization including *P.aeruginosa* [6,14]. This result can be explained by that contracting of bacteria by patient increase with longer period of exposure to micro-organisms present in hospital.

When we considered the association between skin-nasopharyngeal *P.aeruginosa* colonization and type of underlying diseases, there was no association with malignancy (table 4). This result is dissimilar to all reports in which malignancy was a risk factor for colonization bacteria in hospitalized patients [6]. However, there was significant association between *P.aeruginosa* colonization and burn with OR= 45, CI=19-105, p<0.001(table 4). This result is similar to that reported by previous reports [8-13] in which burn was risk factor of *P.aeruginosa* colonization [6-13]. This association can be explained by that original thermal injury creates a breach in the surface of the skin which make it more susceptible to colonization with micro-organisms, and *P. aeruginosa* use quorum sensing to induce the production of virulence factors such as proteases, hemolysins, exotoxins A and pyocyanin which help bacteria to colonized as stated previously in the discussion of Burn ward[14].

We carried this study because of the knowledge of *P.aeruginosa* prevalence and the current antimicrobial profile is necessary in selection of appropriate empirical treatment of these infections and control of *P.aeruginosa* in hospitals is essential. *P.aeruginosa* is a common nosocomial pathogen [15] that causes infections with a high mortality rate[16]. This latter is, in part, attributable to the organism's intrinsically high resistance to many antimicrobials and the development of increased, particularly multidrug resistance in healthcare settings [17], both of which complicate anti-pseudomonal chemotherapy. Indeed, numerous studies point to a link between multidrug resistance and increased morbidity/ mortality, as well as increased length of hospital stay and increased hospital costs [18]. In our study the isolated *P.aeruginosa* was completely resistant (100%), to Aztroneome, Ceftriaxone, and Ciprofloxacine. This result is different from that reported in USA in which the rate of Aztroneome, Ceftriaxone, and Ciprofloxacine resistant was not more than 52.2% [19]. Also the incidence of Aztroneome, Ceftriaxone, and Ciprofloxacine resistant in the European countries has been documented by Lambert *et al*. [16] in which the rates were ranged from 50-72%. These high rates in our study can be explained by the fact that acquisition of resistance genes [e.g., those encoding β-lactamases [20] and aminoglycoside-modifying enzymes [21] via horizontal gene transfer can and do drive antimicrobial/multidrug resistance development in *P. aeruginosa*, more commonly mutations of chromosomal genes (target site, efflux mutations) [22]. The results of our study showed higher rates of resistant of *P. aeruginosa* (83.3-85.7%) to Amikacin, Ampicillin sulbactam, and Levofloxacin. This result is different from study carried out at Canada in 2010 which showed significant variable susceptibility pattern with lower resistance rates to Amikacin, (25%), Ampicillin sulbactam (35%), and Levofloxacin (25%). There were low resistant rates of resistant for our isolated *P.aeruginosa* for Gentamicin (35.7%), Imipenem (11.9%), Pipracillin Tazobactam (11.9%), and Ticaracillin calvulanic acid (31%). The current results are similar to that reported from USA in which resistance rates to these antibiotics were not exciding 30%.[17] Owing to the increased prevalence of multidrug-resistant *P. aeruginosa*, "older" antimicrobials like the polymyxins (polymyxin B and colistin) are back in favor, with earlier issues surrounding nephrotoxicity largely dealt with [23,24]. The rate in our study for Colistin sulphat resistant was 14.3%. This rate is similar to that reported from Russia and Western Europe. [23,24]

Conclusion

The study have been highlighted the role of hospitalization as a significant risk for *P.aeruginosa* colonization; concerted and coordinated efforts are required both in the hospital and community to tackle this. Elimination of health care–associated infections is a priority of the Ministry of Health and

Population. Continued improvements in patient safety depend on maintaining a comprehensive understanding of the epidemiology of health care–associated infections. Currently, no single Yemen surveillance study can provide estimates of the burden of all types of such infections across hospital care patient populations. Effective antimicrobial activity as well as cost effectiveness should be considered in drugs prescribed for *P.aeruginosa* infections. Oral dosing options for antibiotics can allow earlier discharge of hospitalized patients and minimize the chances of *P.aeruginosa* multi-resistant emergence. Good hospital infection control measures prove to be the main stay against these infections because antibiotics can never be an effective alternative to good medical practice.

Limitation

This study has a number of limitations. Some data is missing and the numbers are small. Only skin and nasopharyngeal specimens were collected. In the protocol of the study rectal colonization was concerned, but most patients were refused to give rectal swab so no more than skin and nasopharyngeal specimens were collected. No molecular studies were used to categories samples further, in order to determine cross resistance or resistance mechanisms. Nevertheless, we believe this study adds further information to the epidemiology of a significant pathogen in the hospital setting in Sana'a city. In addition, although we are confident that the studied hospitals are representative of hospitals within Sana'a city, they may not be representative of all Yemen hospitals.

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Conflict of interest:

"No conflict of interest associated with this work".

Author's contribution

This research work is part of A M.Sc. thesis. The candidate is the first author (RTFA) who conducted the laboratory and field works; and wrote up the thesis. The corresponding author (HAA) supervised the laboratory and field works, revised and edited the thesis draft and the manuscript.

References

1-Suárez C, Peña C, Gavaldà L, *et al.* Influence of carbapenem resistance on mortality and the dynamics of mortality in *Pseudomonas aeruginosa* bloodstream infection. Int J Infect Dis 2010;14Suppl 3:e73-8.

2- Magill SS, Edwards JR, Bamberg W, *et al.* Multistate point-prevalence survey of health care-associated infections. N Engl J Med. 2014;370:1198–1208.

3- Thuong M, Arvaniti K, Ruimy R, *et al.* Epidemiology of *Pseudomonas aeruginosa* and risk factors for carriage acquisition in an intensive care unit. J Hosp Infect. 2003; 53:274–282.

4- Nesher L, Rolston KV, Shah DP, *et al.* Fecal colonization and infection with *Pseudomonas aeruginosa* in recipients of allogeneic hematopoietic stem cell transplantation. Transpl Infect Dis 2015;17:33–38.

5- Cheesbrough MC. District Laboratory Practice in Tropical Countries. 1st Edn, Cambridge University Press, Cambridge 2006, UK.

6-Harris AD, Jackson SS, Robinson G *et al.* . *Pseudomonas aeruginosa* colonization in the ICU: Prevalence, risk factors and clinical outcomes. Infect Control Hosp Epidemiol 2016; 37(5): 544–548.

7-Parkins MD, Gregson DB, Pitout JD, *et al.* Population-based study of the epidemiology and the risk factors for *Pseudomonas aeruginosa* bloodstream infection. Infection 2010; 38:25-32.

8-Jain SK, Persaud D, Perl TM, *et al.* "Nosocomial malaria and saline flush". Emerging Infect. Dis 2005; 11(7): 1097–9.

9-Enoch DA, Julie Kuzhively, Andrew Sismey, *et al. Pseudomonas Aeruginosa* Bacteraemia in Two UK District Hospitals. Infect Dis Rep. 2013; 5(1): e4.

10-Cheong HS, Kang CI, Wi YM, *et al*. Inappropriate initial antimicrobial therapy as a risk factor for mortality in patients with community-onset *Pseudomonas aeruginosa* bacteraemia. Eur J Clin Microbiol Infect Dis 2008;27:1219-25.

11-Kang CI, Kim SH, Kim HB, *et al. Pseudomonas aeruginosa* bacteremia: risk factors for mortality and influence of delayed receipt of effective antimicrobial therapy on clinical outcome. Clin Infect Dis 2003;7:745-51.

12- Micek ST, Lloyd AE, Ritchie DJ, *et al. Pseudomonas aeruginosa* bloodstream infection: importance of appropriate initial antimicrobial treatment. Antimicrob Agents Chemother 2005; 49:1306-11.

13-Lyczak J.B., Cannon C.L., Pier G.B. Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist. Microbes and Infection 2000; 2:1051-1060.
14-Van Delden C, Iglewski B H. Cell-to-cell signaling and Pseudomonas aeruginosa infections. Emerging Infecetious Diseases 1998; 4 (4):1-4.
15-Zhanel G G, DeCorby M, Adam H, *et al.* Prevalence of antimicrobial-resistant pathogens in Canadian hospitals: results of the Canadian Ward Surveillance Study (CANWARD 2008). *Antimicrobial agents and Chemotherapy* 2010; *54*(11):4684-4693.
16-Lambert ML, Suetens C, Savey A *et al*. Clinical outcomes of health-care-associated infections and antimicrobial resistance in patients admitted to European intensive-care units: a cohort study. The Lancet infectious diseases 2011; *11*(1):30-38.
17-Keen E F, Robinson B J, Hospenthal D R *et al.* Prevalence of multidrug-resistant organisms recovered at a military burn center. Burns 2010; 36(6):819-825.
18-Tumbarello M, Repetto E, Trecarichi EM *et al*. Multidrug-resistant *Pseudomonas aeruginosa* bloodstream infections: risk factors and mortality. Epidemiology & Infection 2011; 139(11): 1740-1749.
19-Harris AD, Perencevich E, Roghmann M C *et al.* Risk factors for piperacillin-tazobactam-resistant *Pseudomonas aeruginosa* among hospitalized patients. Antimicrobial Agents and chemotherapy *2002;* 46(3):854-858.
20-Zhao WH and Hu Z Q. *β-lactamases* identified in clinical isolates of *Pseudomonas aeruginosa*. Critical reviews in microbiology 2010;36(3):245-258.

21-Ramirez MS and Tolmasky ME. Aminoglycoside modifying enzymes. Drug Resistance Updates 2010; 13(6), 151-171.

22-Strateva T and Yordanov V. *Pseudomonas aeruginosa*-a phenomenon of bacterial resistance. J Med Microb 2009; 58(9):1133-1148.

23-Zavascki A P, Goldani LZ, Li J and Nation RL. Polymyxin B for the treatment of multidrug-resistant pathogens: a critical review. Journal of antimicrobial chemotherapy 2007; 60(6):1206-1215.
24-Molina J, Cordero E and Pachón J. New information about the polymyxin /colistin class of antibiotics. Expert opinion on pharmacotherapy 2009;10(17):2811-2828.

Table 1: The association between skin-nasopharyngeal *pseudomonas aeruginosa* colonization and sex and age of in-patients, Sana'a city, Yemen.

Characters	Positive *P.aeruginosa* colonization		Negative *P.aeruginosa* colonization		OR	CI	χ^2	*P*
	No	%	No	%				
Sex								
Male	33	16.4	168	83.6	2.5	1.1-5.5	5.9	0.01
Female	9	7.1	117	92.9	0.39	0.18-0.8	5.9	0.01
Age groups								
≤19 years	2	14.3	12	85.7	1.1	0.2-5.2	0.02	0.8
20-29 years	4	6.3	60	93.7	0.3	0.1-1.1	3.1	0.07
30-39 years	5	9.6	47	90.4	0.7	0.2-1.8	0.46	0.47
40-49 years	6	10.5	51	89.5	0.7	0.3-1.8	0.27	0.6
≥50 years	25	17.9	115	82.1	2.2	1.1-4.2	5.4	0.01
Total	42	12.8	285	87.2				

Table 2: The association between skin-nasopharyngeal *pseudomonas aeruginosa* colonization and type of wards of in-patients, Sana'a city, Yemen.

Wards	Positive colonization		Negative colonization		OR	CI	χ^2	*P*
	No	%	No	%				
ICU n= 69	9	13	60	87	1	0.41-2.5	0.003	0.95
Burn n=48	30	62.5	18	37.5	37	16.2-84	123	<0.001
Medical wards n=210	3	1.4	207	98.6	0.02	0.008-0.09	68	<0.001
Total n=327	42	12.8	285	87.2				

Table 3: The association between skin-nasopharyngeal *pseudomonas aeruginosa* colonization and duration of hospitalization for in-patients, Sana'a city, Yemen.

Duration	Positive colonization		Negative colonization		OR	CI	χ^2	*P*
	No	%	No	%				
1-7 days n=129	6	4.7	123	95.3	0.2	0.08-0.51	12.7	<0.001
8-16 days n=120	15	12.5	105	87.5	0.9	0.5-1.8	0.02	0.88
≥17 days n=78	21	27	57	73	4.0	2-7.8	18.1	<0.001
Total n= 327	42	12.8	285	86.7				

Table 4: The association between skin-nasopharyngeal *pseudomonas aeruginosa* colonization and underlying diseases for in-patients, Sana'a city, Yemen.

Underlying disease	Positive colonization		Negative colonization		OR	CI	χ^2	P
	No	%	No	%				
Burn n=45	30	66.7	15	33.3	45	19-105	135	<0.001
Cardiovascular diseases n=66	1	1.5	65	98.5	0.08	0.01-0.6	9.4	0.002
Malignancy n=72	8	11.1	64	88.9	0.8	0.3-1.8	0.24	0.61
Operation n=21	1	4.8	20	95.2	0.3	0.04-2.4	1.3	0.25
Liver diseases n=84	1	1.2	83	98.8	0.05	0.008-0.4	13.7	<0.001
Respiratory diseases n=39	1	2.6	38	97.4	0.15	0.02-1.1	4.1	0.04
Total n=327	42	12.8	285	87.2				

Table 5: Antibiotic sensitivity of isolated *pseudomonas aeruginosa*

Antimicrobial agents	**Sensitive**		**Resistance**	
	Number	%	Number	%
Amikacin	6	14.3	36	85.7
Ampicillin sulbactam	7	16.7	35	83.3
Aztroneome	0	0.0	42	100
Ceftriaxone	0	0.0	42	100
Chloramphenicol	3	7.1	39	92.9
Ciprofloxacine	0	0.0	42	100
Gentamicin	27	64.3	15	35.7
Imipenem	37	88.1	5	11.9
Levofloxacin	6	14.3	36	85.7
Netilmicin	12	28.6	30	71.4
Pipracillin Tazobactam	37	88.1	5	11.9
Ticaracillin calvulanic acid	29	69	13	31
Colistin sulphat	36	85.7	6	14.3