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Genotyping of *Pseudomonas aeruginosa* Strains Isolated from Surgical Site Infected Patients by RAPD-PCR

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Abstract

Background: *Pseudomonas aeruginosa* is one of the most important causes of nosocomial infections that easily gains resistance to many antibiotics. This opportunistic pathogen is a major health hazard particularly in immunocomprmied patients and patients with surgical wound infection. *P. aeruginosa* may be originated from different sources and comprises a high colonization and transmission capacity. The aim of this study is to determine the antimicrobial susceptibility pattern of the *P. aeruginosa* isolates and to investigate the potential sources of infection in surgical site infected patients by genotyping using RAPD-PCR.

Methodology: samples were taken from patients (217), health staff (30), environment (88) and antiseptics and hand washing solutions (60). After isolation and identification of *P. aeruginosa*, antimicrobial susceptibility testing was performed using disc diffusion method. DNA was extracted from the isolates and RAPD-PCR method was applied to the DNA extract using a short single primer of 272. The technique created repetitive electrophoresis patterns which was used for genotypic differentiation.

Results: 24.8% of patient samples were positive for *P. aeruginosa* and 10% of staff hand samples were positive. The highest frequency of *pseudomonas* isolation from environmental samples was from mops (55.6%) followed by door handles (44.4%) and sinks (40%). The RAPD typing method gave higher discriminatory index (0.807) than antibiogram (0.617). Epidemiological linkages were proven by analyzing antibiogram and RAPD-PCR typing data.

Conclusion: hand hygiene, environmental cleaning and disinfection of patient objects to reduce environmental reservoirs of *P. aeruginosa*. It is better to limit the usage of antibiotics in the hospitals as ceftolozane- tazobactam and ceftazidime- avibactam to be used only when absolutely needed. RAPD molecular typing method was superior to antibiotic typing and should be used in tracing the source of infection.

Keywords: Hygiene, patients, molecular, Epidemiological, genotyping

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INTRODUCTION

Surgical site infection (SSI) just as other healthcare-associated infections (HCAIs) is a major patient safety concerns in hospitals. It tremendously impacts negatively on the patient's well-being as well as on the health-care personnel and financial resources for managing the condition. Despite the numerous preventive measures recommended for its reduction, SSIs continue to occur among surgical patients with substantial increase in the cost of healthcare, prolonged hospitalisation and jeopardised health outcomes. It is sometimes associated with considerable morbidity and occasional mortality [1]. An approximate of 7–10 additional days on the length of hospital stay has been linked with SSI [2,3]. The health-care cost for patients with SSI has been estimated to be twice that of the patients without SSI [3,4]. Furthermore, an increased relative risk of death and readmission for patients with SSI compared to uninfected patients have been documented [1,5].

Patients with severe infected wound present an immunosuppression condition and consequently higher susceptibility to infections by nosocomial pathogens such as *P. aeruginosa* with a high mortality rate mainly due to the high intrinsic resistance of microorganism to many

antimicrobials[6]. *P. aeruginosa* exhibits intrinsic resistance to a lot of different types of chemotheraopeutic agents and antibiotics [7]. Prevention and control of *P. aeruginosa* infections are still among serious nosocomial problems worldwide. Host immunodeficiency, combined with a high incidence of antibiotic resistance, makes treatment of *P. aeruginosa* infections a serious medical challenge [8].

Understanding pathogen distribution and relatedness is essential for determining the epidemiology of nosocomial infections and aiding in the design of rational pathogen control methods [9]. PCR-based molecular genotyping of *P. aeruginosa* is of major importance in the elucidation of transmission routes that compared to phoentyping methods is less affected by environmental factors. In general, molecular methods comprises higher discriminatory power and higher reproducibility than phenotypic tests becouse of their ability to detect minor genome differences and the higher stability of molecular targets compared with that of phenotypic profiles for some species [10].

Different genotypic systems have been described for characterizing *P. aeruginosa* isolates. These systems include hybridization with specific probes and pulse-field gel electrophoresis (PFGE) known as gold standard genotypic technique, and have proved to have good specificity and sensitivity. However, their disadvantages are that they are expensive and time-consuming [11].

Among PCR-based genotyping methods, Random Amplified Polymorphic DNA(RAPD) – PCR has received considerable attention in recent years for epidemiological studies, due to its simplicity, rapidity, sensitivity, reproducibility, low cost (no need to expensive specialized instrumentations). It comprises high strain differentiation power and is a definitive method for molecular characterizing of bacteria [12]. This Rapid methodology has been shown to be as discriminatory as PFGE for typing *P. aeruginosa* and was recommended for the primary screening of large numbers of isolates because of its efficiency [13, 14].

RAPD reactions are PCR reactions, but use a short (8–12mers) single primer, that could attach randomly to the several DNA sequences in the bacterial genome and create repetitive electrophoresis patterns which is used for genotypic differentiation. If a mutation has occurred in the template DNA at the site that was previously complementary to the primer, a PCR product will not be produced, resulting in a different pattern of amplified DNA Segments on the gel. The other difference between this method and ordinary PCR is using of two different annealing tempratures [15].

Since *P. aeruginosa* is a major cause of nosocomial infections, particularly in infected wound patients, so the knowledge of the spread of *P. aeruginosa* strains is of epidemiological importance in order to monnitor the spreading of the strains [16].

The aim of this study was then to determine the antimicrobial susceptibility pattern of the *P. aeruginosa* isolates and to investigate the potential sources of infection in surgical site infected patients by genotyping using RAPD-PCR.

METHODOLOGY

This study was conducted in the Medical Microbiology and Immunology Department and General Surgery Department, Faculty of Medicine, Zagazig University in the period from December 2017 to March 2020. This study was approved by the ethics committee of Zagazig University Hospitals.

Study sample

A total of 395 samples were collected from patients, staff hands, the environment and antiseptics and hand washing solutions from General Surgery Department of Zagazig University hospitals:

- 217 samples were collected from postsurgical septic wound, exudate were collected using sterile cotton swabs by introducing them deeply into the depth of the infected wounds [17].
- 30 samples were collected from staff hands at midday, by which time staff members had been in contact with patients for several hours [18].
- 88 environmental samples were taken throughout the department, concentrating on damp surfaces and areas with maximum potential for cross infection (dressing trolleys, bed rails, over beds, door handles, sinks, tables tops and mops). Surfaces were swabbed with sterile cotton swab sticks, which were used to inoculate nutrient broth tubes. Fluid medium was selected as primary culture medium to dilute disinfectants and encourage growth of low organism numbers. After 24h incubation subcultures were made in agar plates and incubated at 37°C [19].
- 60 Fluid samples such as antiseptics and hand washing solutions were pipetted using sterile syringes. One ml of the antiseptics and hand washing solutions samples were added to test tubes containing 9 ml sterile tryptic soy broth and incubated at 37°C for 24 hours. 0.01 ml of the diluted samples were sub-cultured on agar plates and incubated aerobically at 37°C for 24 hours[20].

Isolation and identification of *P. aeruginosa*

All samples were cultured on the nutrient, MacConkey and blood agar plates. *P. aeruginosa* strains were isolated and identified based on standard microbiology techniques [21]. *P. aeruginosa* isolates were preserved at -70°C in Trypticase Soy Broth medium (Himedia, India) supplemented 15% glycerol until further processing.

Antibiogram

Antibiotic resistance testing was done by the Kirby–Bauer disk diffusion method according to Clinical Laboratory Standards Institute guidelines [22,23]. The following antibiotics (Oxoid, UK& Liofilchem, Italy) were used: ceftazidime 30 µg, cefepime 30 µg, gentamicin 10 µg, tobramycin 10 µg, amikacin 30 µg, imipenem 10 µg, meropenem 10 µg, ciprofloxacin 5 µg, levofloxacin 5 µg, aztreonam 30 µg, piperacillin-tazobactam 100/10 µg, ceftazidime-avibactam 30/20 µg, ceftolozane-tazobactam 30/10 µg and colistin 10 µg.

PCR methods

DNA extraction

All strains were freshly cultured on nutrient agar before DNA extraction using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. The extracts were then kept at –20 °C until use.

RAPD-PCR typing

RAPD-PCR was performed as described previously [13]. In brief, DNA amplification was performed on a thermo cycler (Biometra, Germany) in a final volume of 25 µl containing 12.5µl Taq PCR Master Mix, (2µl) of 272 primer (5′ AGCGGGCCAA 3′) [24] (Thermo Fisher Scientific, USA), double distilled water (8.5µl) and 2µl genomic DNA. The cycling conditions were as follows: Initial denaturation at 96°C For 2 min followed by 3 cycles of denaturation at 94°C for 1 min, annealing at 36°C for 2 min, extension at 72°C for 2 min and 29 more cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1

min, extension at 72°C for 1 min and a final extension at 72°C for 5 min.

DNA detection by agarose gel electrophoresis

Amplification products and 1 kilobase DNA molecular weight marker (Enzynomics, Korea) were detected by using agarose gel electrophoresis [25]. These were visualized on a UV transilluminator (Cole-Parmer, USA) and photographed. Genotypes were assigned on the basis of number and weight of band differences [26].

Statistical analysis

All data were tabulated and then processed using SPSS, version 25. Qualitative variables were expressed by percentages and compared using the chi-squared test. A P-value < 0.05 was considered statistically significant. The numerical discriminatory index, which is a measure of the discriminatory ability of the typing methods was calculated according to Hunter [27].

RESULTS

Isolation rate of *P. aeruginosa*

P. aeruginosa was isolated from 24.8% of patient samples, 10% of staff hand samples, 19.3% of environmental samples and 5% from antiseptic and hand washing solution samples (Table 1).

The highest rate of *P. aeruginosa* which was identified from hospital environmental specimens in this study were from mops 5/9 (55.6%) followed by door handles 4/9 (44.4%) and sinks 4/10 (40%), this result could be explained by the fact that bacteria grow very well at sites with adequate amount of moisture and where people commonly come in contact with, while the result showed the least rate of *P. aeruginosa* 1/15 (6.7%) in the dressing trolleys and patients' over beds followed with bed rails 2/15 (13.3%) and not detected in tables tops, which are mostly kept dry.

Antibiotic resistance

The 77 *P. aeruginosa* isolates were mostly sensitive to ceftolozane-tazobactam (93.5%), ceftazidime-avibactam (90.9%), colistin (89.6%), meropenem (76.6%), imipenem (71.4%), piperacillin-tazobactam (67.5) and amikacin (63.6%) followed by tobramycin (55.8%), gentamicin (54.5%), ciprofloxacin (51.9%) and levofloxacin (48.1%). On the other hand, they were mostly resistant to ceftazidime and aztreonam (71.4%) and (57.1%) of isolates were resistant to cefepime (Table 2).

Table 1. Frequency of *Pseudomonas aeruginosa* isolates among patients, staff, hospital environmental and antiseptic and hand washing solution samples.

Sample types	Sample no.	*P. aeruginosa* isolates	
		No.	(%)
Patient samples	217	54	24.8
Staff samples	30	3	10.0
Environmental samples	88	17	19.3
Antiseptic and hand washing solution samples	60	3	5.0
Total	395	77	19.5

Table 2. Antibiotic resistance pattern of *P. aeruginosa* isolates (n=77).

	Resistant		Intermediate		Sensitive	
	No.	%	No.	%	No.	%
Ceftazidime	55	71.4	0	0.0	22	28.6
Cefepime	44	57.1	12	15.6	21	27.3
Gentamicin	32	41.6	3	3.9	42	54.5
Tobramycin	34	44.2	0	0.0	43	55.8
Amikacin	28	36.4	0	0.0	49	63.6
Imipenem	22	28.6	0	0.0	55	71.4
Meropenem	18	23.4	0	0.0	59	76.6
Ciprofloxacin	29	37.7	8	10.4	40	51.9
Levofloxacin	37	48.1	3	3.9	37	48.1
Aztreonam	55	71.4	0	0.0	22	28.6
Piperacillin-tazobactam	25	32.5	0	0.0	52	67.5
Ceftazidime- avibactam	7	9.1	0	0.0	70	90.9
Ceftolozane- tazobactam	5	6.5	0	0.0	72	93.5
Colistin	8	10.4	0	0.0	69	89.6

Antibiotyping

The isolated *P. aeruginosa* strains were grouped into 11 patterns that were designated A1-A11 according to their results of antibiotic susceptibility. Most of the patterns (A3, A4 and from A6 to A11) showed multidrug resistance (MDR) as strains were non-susceptible to one or more agents in three or more antimicrobial categories. Number of isolates belonging to the most numerous pattern (A7) was 23 (29.9%) strains. On the other hand, number of strains belonging to the least numerous patterns (A8 and A10) were 2 (2.6%) strains (Table 3).

RAPD-PCR typing

P. aeruginosa isolates were typeable by RAPD-PCR and yielded 15 RAPD patterns with 2 to 7 amplification bands. The size of amplified DNA bands ranged from 180 bp to 2700 bp (Figure 1)

On calculating the discriminatory index of both typing methods (antibiotyping and RAPD), we found that RAPD typing gave a higher discriminatory index (0.807) than the antibiogram (0.617) (Table 4).

Table 3. Observed patterns of antibiotic susceptibility for isolated *P. aeruginosa* strains (n=77).

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
CAZ	S	R	R	R	S	S	R	R	R	R	R
FEP	S	R	S	M	S	M	R	S	R	R	R
CN	R	S	S	S	S	S	R	R	S	S	M
TOB	S	S	S	R	R	S	R	S	S	S	S
AK	S	S	R	S	S	R	S	R	R	S	S
IPM	S	S	S	R	S	S	S	S	R	R	R
MEM	S	S	S	S	S	S	S	S	R	R	R
CIP	R	S	S	S	R	M	S	R	R	S	S
LEV	S	S	S	R	S	R	R	R	S	S	M
ATM	S	R	R	R	S	S	R	R	R	R	R
PTZ	S	S	R	S	S	S	S	R	R	R	R
CZA	S	S	S	S	S	S	S	R	S	R	R
C/T	S	S	S	S	S	S	S	R	S	S	R
CT	S	S	S	S	S	R	S	S	S	S	S
N (%)	7 (9.1)	3 (3.9)	5 (6.5)	4 (5.2)	7 (9.1)	8 (10.4)	23 (29.9)	2 (2.6)	13 (16.9)	2 (2.6)	3 (3.9)

Figure 1. Different observed RAPD-PCR patterns for isolated *P. aeruginosa* strains.

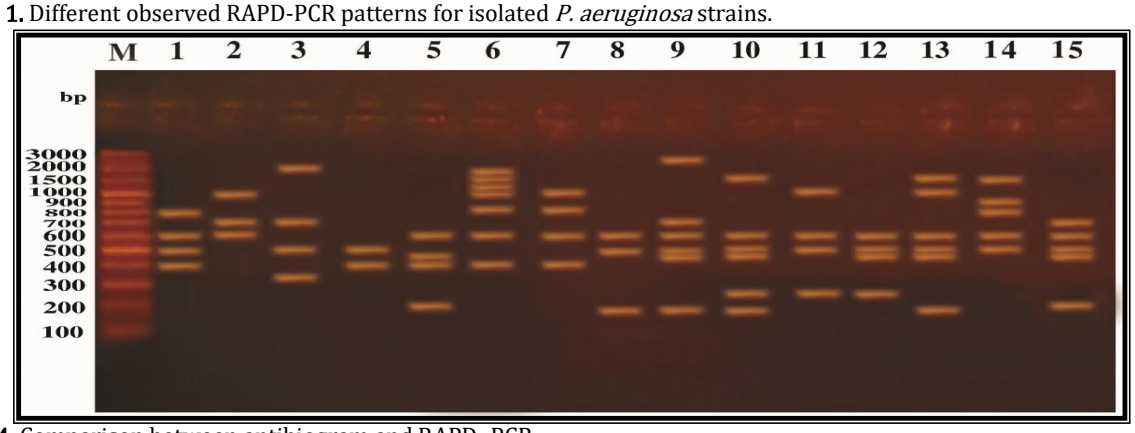

Table 4. Comparison between antibiogram and RAPD- PCR.

	No. of different patterns	No. of strains belonging to the most numerous pattern	Numerical discriminatory index
Antibiogram	11	23	0.617
RAPD- PCR	15	17	0.807

There are possible epidemiological relationships proved by analyzing antibiogram and RAPD-PCR typing data among different sites of *P. aeruginosa* isolation (Table 5).

Table 5. Epidemiological analysis of typing data.

Isolates	Source	Antibiotic pattern	RAPD Pattern
e11	Bed rail	A1	I
e12	Door handle	A1	I
h3	Hand	A1	I
p7, p14, p15, p42	Patient	A1	II
p26, p28, p41	Patient	A2	III
p25, p29, p34, p46, p48	Patient	A3	IV
p6, p12, p39, p53	Patient	A4	V
p30, p33, p44, p45, p49	Patient	A5	VI
e14	Dressing trolley	A5	VI
s1	Antiseptic solution	A5	VI
p17, p22	Patient	A6	VII
p27, p51, p52	Patient	A6	VIII
e8	Sink	A6	VIII
e13	Mop	A6	VIII
e16	Door handle	A6	VIII
p4, p8, p10, p19, p31, p32, p35, p36, p37, p38, p40, p43, p47	Patient	A7	IX
e9	Sink	A7	IX
e15	Mop	A7	IX
s2	Hand washing solution	A7	IX
h2	Hand	A7	IX
p9, p50, p54	Patient	A7	X
e5	Bed rail	A7	X
e2	Over bed	A7	X
h1	Hand	A7	X
e1	Sink	A8	XI
e4	Door handle	A8	XI
p5, p11, p13, p16	Patient	A9	XII
p1, p2, p3, p18, p21	Patient	A9	XIII
e3	Sink	A9	XIII
e7	Door handle	A9	XIII
e6	Mop	A9	XIII
s3	Hand washing solution	A9	XIII
e10, e17	Mop	A10	XIV
p20, p23, p24	Patient	A11	XV

p: patient sample; e: environmental swab; s: solution sample; h: hand swab of staff member

DISCUSSION

Pseudomonas aeruginosa is a common pathogen that causes nosocomial infections particulary surgical site infections [28]. Resistant *P. aeruginosa* is an emerging threat to patients, so clinical laboratories must focus more and more on the epidemiology of hospital-acquired infections. Strain typing is an extremely useful tool in tracking the spread of nosocomial infections [29].

Evidence-based prevention strategies targeting specific pathogens should be based on thorough knowledge of their epidemiology, reservoirs in the hospitals and mode of transmission [30].

Isolating *Pseudomonas aeruginosa* in itself is not enough to conclude epidemiological importance of the site of isolation, so strain typing can increase the efficiency to control the infection by finding out actual sources of patient infections and finding out sources that contain environmental pseudomonads that may receive less stringent treatment.

In our study, we try to detect the epidemiology of *P. aeruginosa* in the General Surgery Department. Several patients, staff hands, environmental, antiseptics and hand washing solutions samples have been typed using RAPD - PCR to investigate possible relationships. Antibiogram was performed as a typing method.

In the present study, *P. aeruginosa* strains were isolated from 24.8% of the surgical wound infected patients' samples. This in agreement with rate reported by Akhi et al [31] in Iran (26%) and pramila et al [32] in India (26%). Our ratio is relatively high when compared with other studies, In Egypt Raafat et al [33] isolated the organism from 17.8% of samples, in Baghdad, Iraq, Al-Kadhmi et al [34] isolated it from 14.6% of samples, in Iraq Al-Zaidi [35] isolated it from 19.5% of samples and in India Bastola et al [36] and Mundhada et al [37] isolated it from 15.4% and 18.9% respectively. A study done in Saudi Arabian found that *P. aeruginosa* was the most frequently isolated bacteria (31.6%) from wound infections [38]. The possible reason for these varied findings with other studies could be due to populations; different surgical procedures and protocol used for disinfection and antisepsis as well as timing of specimen collections.

In this study 30 samples of staff hands were cultured, three of them (10%) were positive for *P. aeruginosa*. Mansour et al [39] in Egypt reported similar results. Ghonaim and Nada [40] in Egypt also showed similar results, as they reported that 2 (11%) out of 18 staff hands samples were positive for *P. aeruginosa*. Our result is higher than that reported (3.5%) by Crivaro et al [41] and 2 (6.7%) out of 30 staff hands samples by Mansour et al [39] in Saudi Arabia. The higher percentage in this work could be due to lack of compliance of health care workers to hand washing practice inspite of presence of adequate facility for it or larger investigated samples.

In the present study the environmental sampling showed that 17 out of 88 (19.3%) of the samples were positive for *P. aeruginosa*. This agrees with Gad et al [42] and Afifi et al [43] in Egypt who isolated *P. aeruginosa* from (19.5%) and 42/200 (21%) of hospital environmental samples respectively. Al-Zaidi [35] in Iraq isolated *P. aeruginosa* from 11/50 (22%) of hospital environmental samples and Karami et al [44] in Iran reported that the prevalence rate of *P. aeruginosa* isolated from hospital environmental samples was 20/108 (18.5%). Our result is less than that reported in other studies; 15/60 (25%) and 14/60 (23.3%) in Egypt and Saudi Arabia by Mansour et al [39] and 30/100 (30%) in Egypt by Ghonaim and Nada [40]. This reflects the fact that *P. aeruginosa* is ubiquitous in the hospital environment. While in the study of Phoon et al [45] the incidence of *P. aeruginosa* in the hospital environmental isolates was 5.1%. This difference in prevalence rate among several studies can be attributed to differences in hygienic strategies, infection control procedures and geographical location.

The isolation rate was the highest from mops 5/9 (55.6%) followed by door handles 4/9 (44.4%) and sinks 4/10 (40%). This result could be explained by the fact that bacteria grow very well at sites with adequate amount of moisture and where people commonly come in contact with. One out of thirty samples (3.3%) of antiseptic solutions and two out of thirty samples (6.7%) of hand washing solutions were contaminated by *P. aeruginosa*.

Antibiotic resistance rates were highest to ceftazidime (71.4%), aztreonam (71.4%) and cefepime (57.1%). The obtained findings are probably due to the wide use of these antibiotics in the investigated department. In agreement with this result, Abaza et al [46] in Egypt recorded a high level of resistance to ceftazidime 80%, aztreonam 85.7% and cefepime 79.4%. Another study in Egypt also showed a resistance rate of 68.1% to each of ceftazidime and cefepime and 57.4% to azetronam by Elmaraghy et al [47]. In contrast, El-Ageery and Al Otibi [38] in Saudi Arabia, reported that 33.3% of isolates were resistant to ceftazidime and cefepime and 29.6% were resistant to aztreonam. This difference could be attributed to the different rate of use of these antibiotics in different localities. The lowest rate of resistance in the current study was to ceftazidime-avibactam and ceftolozane- tazobactam 9.1% and 6.5% respectively making them the last resort of therapy. Similarly Liao et al. [48] in Taiwan found 9% and 7% resistance rate to ceftazidime-avibactam and ceftolozane-tazobactam respectively.

On calculating the numerical discriminatory (D) index of both typing methods (antibiotyping and RAPD), we found that RAPD typing gave a higher discriminatory index (0.807) than the antibiogram (0.617). This is in agreement with Freitas and Barth [49] who declared that the low discriminatory power of susceptibility tests was not surprising since the power of method was determined by the number of types defined by it and the relative frequencies of these types.

By analyzing various typing data, we found possible epidemiological linkages; sharing of certain RAPD patterns among patient strains may be explained by horizontal transmission from patient to another patient, probably from the hands of staff members or environmental sources.

Our results found that the patient isolates belong to RAPD genotypes II to X and XII, XIII and XV. Environmental isolates belong to RAPD genotypes I, VI, VIII, IX, X, XI, XIII and XIV. Genotypes VI, VIII, IX, X and XIII contain both environmental and patient isolates.

Environmental contamination may contribute to transmission of healthcare pathogens when health care workers contaminate their hands or gloves by touching contaminated surfaces, or when patients come into direct contact with contaminated surfaces. Environment may serve as a reservoir for *P. aeruginosa*. *P. aeruginosa* can survive at least 3-6 months on dried blood or cotton and as long as four weeks on other surfaces, and it is

particularly well adapted to wet or damp conditions [50].

A direct link among one hand strain, thirteen patients' strains, belonging to RAPD (IX) genotype was proven. In addition, a direct link among one hand strain and three patients' strains, belonging to RAPD (X) genotype was proven. Other studies revealed that *P. aeruginosa* was isolated from hands of health staff, but molecular typing methods failed to show direct link with strains isolated from patients [40]. This discrepancy of results is may be due to small staff sample size.

Mops, door handles and sinks had a central role in the spread of *P. aeruginosa* in the general surgery department. Epidemiological linkage was proven among patients and door handles by harboring strains belonging to RAPD (VIII) and RAPD (XIII) genotypes. This might be explained by inadequate application of standard precautions for infection control.

Regarding linkage among patients and mops, both of them harbored strains belonging to RAPD (VIII), RAPD (IX) and RAPD (XIII) genotypes. It may be explained by that, in the department included in our study, the mops are used to clean the wards as well as bathrooms. Bathrooms, being humid environment, are common reservoir of *P. aeruginosa*.

Epidemiological linkage was also proven among patients and sinks by harboring strains belonging to RAPD (VIII), RAPD (IX) and RAPD (XIII) genotypes. This could be possibly explained by that, *P. aeruginosa* survives well in the hospital environment in damp areas such as sinks and taps, backsplash and aerosols may contaminate hands during hand washing with subsequent transmission to patients through contact.

There is also epidemiological linkage was proven among patients, antiseptic solution and dressing trolley by harboring strains belonging to RAPD (VI) genotype. Evacuation of antiseptic solution into the containers is a possible reason that could explain its linkage to dressing trolley by harboring strains belonging to RAPD (VI) genotype.

Another epidemiological linkage was proven among patients and hand washing solutions by harboring strains belonging to RAPD (IX) and RAPD (XIII) genotypes.

Lastly we recommend hand hygiene, environmental cleaning and disinfection of patient objects to reduce environmental reservoirs of *P. aeruginosa*. Reducing the usage of antibiotics in the hospitals as ceftolozane-tazobactam and ceftazidime- avibactam to be used only when absolutely needed. RAPD molecular typing method was superior to antibiotic typing and should be used in tracing the source of infection.

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