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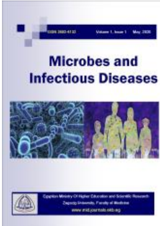

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Original article

Emergence of colistin-resistant *Pseudomonas aeruginosa* in Sohag University Hospitals, Egypt

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ABSTRACT

Background: *Pseudomonas aeruginosa (P. aeruginosa)* is a globally recognized cause of healthcare-associated infections (HAIs), the recent increase of the MDR and XDR *P. aeruginosa* strains encouraged the use polymyxins as a treatment option, and thus the emergence of colistin-resistant strain is an alarming problem. **Objectives:** This study aimed to trace the emergence of colistin-resistance in *P. aeruginosa* strains associated with HAIs in Sohag University Hospitals, to identify the genetic basis of colistin-resistance in these isolates. **Methods:** *P. aeruginosa* strains were isolated and identified phenotypically and genotypically, antibiotic susceptibility of the isolates was tested by disc-diffusion method. The MIC of colistin was measured by E test in colistin resistant isolates. Conventional PCR was used to detect plasmid genes responsible for colistin resistance among the isolates. **Results:** Seventy-six (76%) of *P. aeruginosa* isolates were resistant to colistin, the highest percentage of colistin resistant strains were isolated from patients admitted to General Surgery Department that was (50%), no colistin resistant strains were isolated from patients admitted to Vascular Surgery Department. Colistin-resistant isolates exhibited the highest resistance to polymyxin B, norfloxacin, ofloxacin and gatifloxacin by a percentage of (100%). *mcr-1*gene was detected in (44.4%) of colistin-resistant isolates and *mcr-2* gene in (16.6%). Sensitivity of E-test in comparison with PCR was (100%) and specificity was (86.36%). **Conclusion:** The emergence of colistin resistance in *P. aeruginosa* in our health care setting is an alarming issue that needs strict adherence to the infection control guidelines specially plasmid mediated resistance as it usually associated with MDR and XDR patterns.

Introduction

Pseudomonas aeruginosa (P. aeruginosa) is a globally recognized cause of healthcare-associated infections (HAIs), it has a prevalence of 7.1%–7.3% amongst all HAIs. The most common site of *P. aeruginosa* infection is pneumonia, *P. aeruginosa* accounts for 10%–20% of isolates in cases of ventillator associated pneumonia (VAP) in ICUs showing increasing trends of incidence and mortality rates [1].

This pathogen is responsible for many other severe infections which are difficult to manage, like bacteraemia, complicated intra-abdominal, urinary tract, and surgical site infections (SSIs) mainly in immune-compromised patients with poor prognosis [2]. *Pseudomonas aeruginosa* is the most common Gram-negative organism leading to infection in burn patients, and it is associated with sepsis and death [1].

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Generally, the main mechanisms of antibiotic resistance in *P. aeruginosa* are classified into intrinsic, acquired and adaptive resistance-mechanisms. The intrinsic resistance of *P. aeruginosa* utilizes decreased outer membrane permeability, the use of efflux pumps that expel antibiotics outside the cell, and the secretion of antibiotic- inactivating enzymes. The acquired resistance of *P. aeruginosa* is achieved through either horizontal transfer of resistance genes or mutational changes; also adaptive resistance involves biofilm-formation that serves as a physical barrier to limit antibiotic entrance into the bacterial cell [3].

The recent rise in rates of infections caused by multidrug resistant-*P. aeruginosa*, especially those resistant to carbapenems, has encouraged the use polymyxins as a last resort treatment option. Polymyxins are classes of non-ribosomal cyclic antibiotics which include five chemically distinguished compounds (polymyxins A, B, C, D, and E) of which polymyxin B and colistin (polymyxin E) are the only two polymyxins currently available on the market [4].

Colistin has a remarkable activity against Gram-negative bacteria and it targets the lipopolysaccharide in the outer membrane and is considered the agent of last resort in treatment of infections by MDR Gram-negative bacilli, especially carbapenemase-producing enterobacteriaceae, *P. aeruginosa*, and *Acinetobacter baumannii* [5]. The World Health Organization (WHO) and other government agencies such as Health Canada have re-classified colistin in the category of "very high importance for Human Medicine" [6].

Resistance to colistin is mediated mainly through lipid A structural adjustments, resulting from the addition of phosphoethanolamine (Pet) and 4-amino-4-deoxy-L-arabinose (L-Ara-N) to the lipid A moiety on the surface membrane; these additions make lipid A less cationic such that the anionic colistin is unable to bind and initiate membrane lysis [7].

Till 2016, all traced colistin-resistance mechanisms were attributed to chromosomal genes. Plasmid-mediated colistin-resistance was reported for the first time in China in 2016 [8].The plasmid-mediated colistin resistance genes (*mcr* genes) reduce the bacterial affinity to colistin through encoding phosphoryl-ethanolamine transferase, which reduces the negative charge of the microbial outer membrane, resulting in the development of microbial resistance [9].

This study aimed to trace the emergence of colistin-resistance in *P. aeruginasa* strains associated with HAIs in Sohag University Hositals, to identify the risk determinants responsible this problem, and also to identify the genetic basis of colistin-resistance in these isolates.

Patients and Methods

The study was conducted at the Department of Medical Microbiology and Immunology, Sohag Faculty of Medicine and Sohag University Hospitals and extended for 2 years May 2020 to May 2022.The study included patients developed HAIs after admission to different departments of Sohag University Hospitals like; surgical site infections (SSIs), Urinary tract infections (UTIs), diabetic foot ulcer, VAP, infected burns, chronic chest infections, and infection on-top of cystic fibrosis of the lung.

Samples were taken with consideration of complete aseptic precautions. For pus samples, sterile cotton swabs were used, dry sterile well-closed plastic cups for urine, sputum, and endotracheal aspirate samples. Urine, sputum and endotracheal aspirates were divided into two parts, the first part was centrifuged at 3000 rpm for 10 min. and the deposit was stained by Gram stain (*P. aeruginosa* is a Gram-negative coccobacilli arranged singly, in pairs or in short chains). The other part of the sample was vortexed and cultured on cetrimide culture medium for selective isolation of *P. aeruginosa*, calibrated 10µl loop was used for urine cultures (more than 10^5 CFUs per 1ml of fresh un-centrifuged urine was considered for diagnosis of UTI). Pus samples were enriched with nutrient broth for 24 hours at 37ºC before inoculation on cetrimide agar. Oxidase, catalase, and citrate utilization tests were used for confirmation of identification **(Figure 1a, b, c, and d)**

Molecular characterization of *P. aeruginosa* spp. by detection of *toxA* gene:

All isolates were subjected to PCR to detect *toxA gene* that is unique to *P. aeruginosa* species [10]. A single colony was picked from a freshly streaked cetrimide agar plate to inoculate 1-5 mL of nutrient broth medium. Incubation was done for 12-16 hours at 37°C while shaking at 200-250 rpm. The bacterial culture was harvested by centrifugation at 8000 rpm (6800 × g) in a micro-centrifuge for 2 min at room temperature. The

supernatant was decanted and the remaining medium was removed. DNA was extracted from the isolates by the use of Gene JET Genomic DNA Purification Kit according to the manufacturer's instructions (Thermo Fisher scientific, California). PCR assay was run using the primer toxA gene (toxA-F 5′-CTGCGCGGGTCTATGTGCC-3′ and toxA-R 5'-TGG ATT GCA CTT CAT CTT GG-3'). PCR were carried out in 25 µl reaction volumes containing 12.5 µl PCR master mix, 4.5 µl DNA grade water, 2 µl of each primer and 4 µl of the extracted DNA was added. In each set of experiments, a negative control was included. The negative control was prepared by replacing the DNA template with PCR grade water.

Amplification of the sample according to the following directions (Amplification cycle profile), by using a Biometra thermal cycler (T Gradient software PCR system version 4 - Biometra Whatman company, Goettingen, Germany). The PCR amplification cycling 94°C for 5 min, and then 35 cycles at 94°C for 1 min, 52°C for 45 s, and 72°C for 30 s, followed by a final extension at 72°C for 7 min [10] **(Figure 2)**.

Antibiotic susceptibility testing of *P. aeruginosa* isolates

Susceptibility of *P. aeruginosa isolates* to different antibiotics was tested by the disc diffusion method (Modified Kirby- Bauer method) according to the Clinical Laboratory Standards Institute (CLSI) guidelines [11]. The tested antibiotics included piperacillin (100µg), ceftazidime (30µg), cefepime (30µg), aztreonam (30µg), imipenem (10µg), meropenem (10µg), colistin (10 µg), polymyxin b (300 units), gentamicin (10µg), tobramycin (10µg), amikacin (30µg), netilmicin (30ug), ciprofloxacin (5µg), levofloxacin (5ug), lomefloxacin (10 ug), ofloxacin (5µg), norfloxacin (10µg), gatifloxacin (5µg) (Oxoid Ltd., Basingstoke UK).

Colistin minimal inhibitory concentration (MIC) measurement by E test

Isolates exhibited colistin-resistance by disk diffusion method were further tested for determination of MIC to colistin using epsilometer test (E test) (Oxoid Ltd., Basingstoke UK). Mulller Hinton medium was inoculated by a bacterial suspension equivilant to the turbidity standard of 0.5 McFarland which is equivalent to 1.5×10^8 CFU/ mL. Colistin E test strips were placed on the surface of the inoculum lawn. After overnight incubation, the plate was examined and the border of growth inhibition intersected the E-strip was taken as the MIC. The MIC interpretive criteria provided in CLSI guidelines were used to assign the category of susceptible (≤ 2 µg/mL), intermediate (2-4 µg/mL) or resistant (≥ 4 µg/mL) isolates **(Figure 3)**.

Molecular detection of plasmid-mediated colistin rsistance (*mcr-1* and *mcr-2* genes)

Plasmid-mediated colistin resistance was investigated in all isolates by polymerase chain reaction (PCR) through the detection of *mcr-1* and *mcr-2* genes.

Sample treatment & DNA extraction

According to the manufacturer's instructions, plasmid-DNA was extracted by the use of GeneJET Plasmid Miniprep Kit (Thermo Fisher scientific, California).

Primer: Oligonucleotide primer sequences were used (metabion international AG, Germany). The 2 oligonucleotide primers used for the amplification of a 320 bp-PCR fragments for detection of *mcr-1* gene; Primer A1 *(mcr-1)* F: 5′-AGT CCG TTT GTT CTT GTG GCA-3′. Primer A2 *(mcr-1)* R: 5′-AGA T CC TTG GTC TCG GCT TGA-3′ [12].

The 2 oligonucleotide primers B1 and B2 used for the amplification of a 715 -bp PCR fragments for detection of *mcr-2* gene; Primer B1 *(mcr-2)*F1: 5′-ATG AC A TCA CAT CAC TCT TGG-3′. Primer B2 *(mcr-2)* R: 5′-TTA CTG GAT AAA T GC CGC GCA-3′ [13].

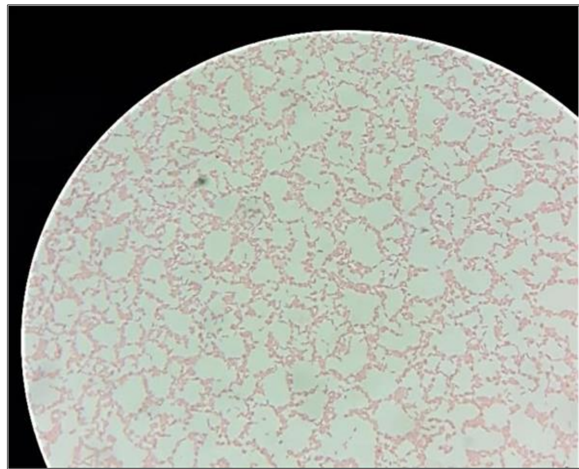
PCR: In a sterile thermal cycler 0.5ml tube, 25 µl PCR reaction mix containing 12.5 µl PCR master mix, 5 µl PCR grade water, 1.25 µl of each primer and 5 µl of the extracted DNA sample was added. In each set of experiments, a negative control was included by replacing the DNA template in the reaction with PCR grade water.

Amplification of the sample was done by Biometra thermal cycler (T Gradient software PCR system version 4 - Biometra Whatman Company, Goettingen, Germany). The PCR amplification cycling profile of *mcr-1* gene was 5 min of denaturation at 94°C (1 cycle), followed by 35 cycles of amplification; each of heat denaturation at 94 °C for 60 s, primer annealing at 44 °C for 30 s, and DNA extension at 72 °C for 30 s then one cycle for final extension at 72°C for 5 minutes. The PCR amplification cycling profile of *mcr-2* gene was 5 min of denaturation at 94°C (1 cycle), followed by 35 cycles of amplification; each of heat denaturation at 94 °C for 60 s, primer annealing at 40 °C for 30 s,

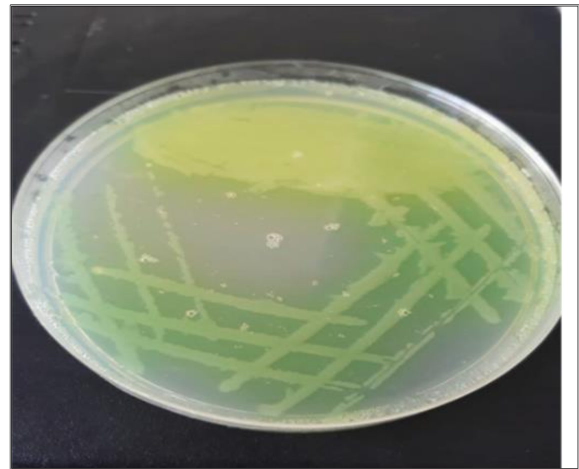
and DNA extension at 72 °C for 40 s then one cycle for final extension at 72°C for 5 minutes [10]. The DNA amplicon was subjected to electrophoresis using 1.5% agarose gel electrophoresis (Electrophoresis power supply-Biometra Whatman Company, Goettingen, Germany), stained with ethidium bromide, and visualized under UV transillumination (**Figures 4, 5**).

Statistical analysis

Data was analyzed using STATA version 14.2 (Stata Statistical Software: Release 14.2 College Station, TX: StataCorp LP.). Quantitative data was represented as mean, standard deviation, median and range. Data was analyzed using Mann-Whitney test. Qualitative data was presented as number and percentage and compared using either Chi square test or fisher exact test. Sensitivity, specificity, positive predicted value and negative predictive value were also calculated. Graphs were produced by using Excel or STATA program. *P* value was considered significant if it was less than 0.05.



a)



b)

c)

d)

Figure 1. a) Direct stained smear, *P. aeruginosa* appear as Gram negative cocco-bacilli, b) Yellow green colonies of *P. aeruginosa* on Cetrimide agar. c) Positive oxidase test for *P. aeruginosa,* d) Positive catalase test of *P. aeruginosa.*

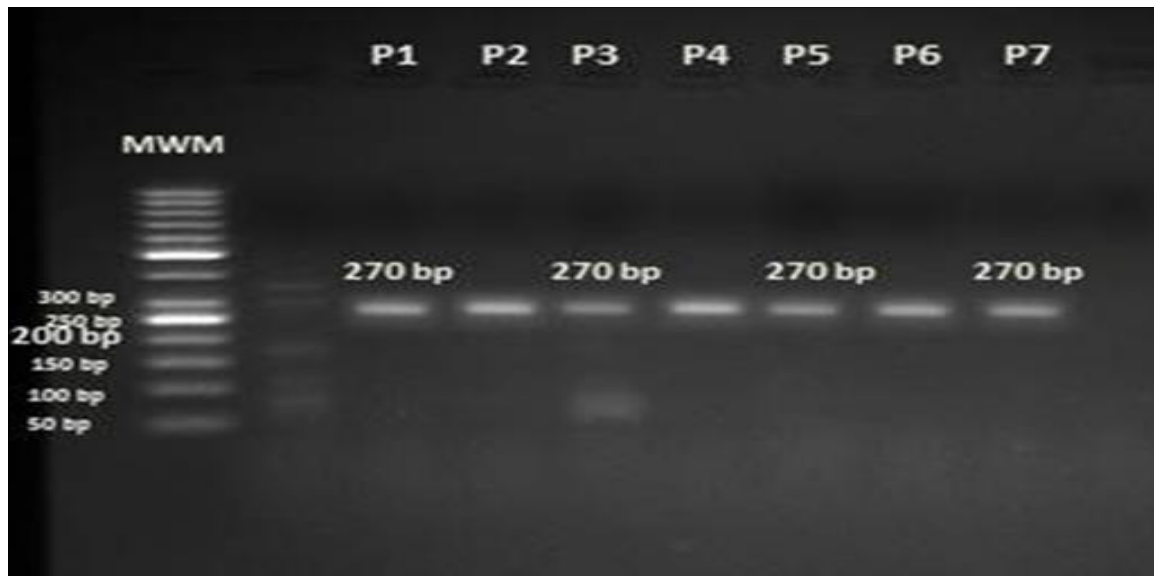
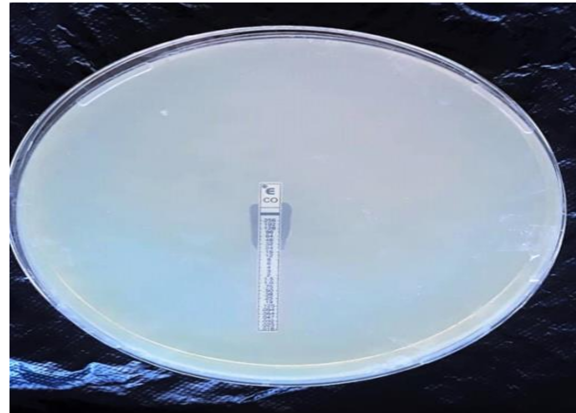


Figure 2. Agarose gel electrophoresis of PCR products after amplification of *toxA* gene. MWM; molecular weight marker (50 bp DNA ladder, DL004, Biomatik), p1, p2, p3, p4, p5, p6 and p7; *P. aeruginosa positive for toxA* gene (MW; 270 bp).



a) **b)**

Figure 3. a) MIC measurement by E test method for P. aeruginosa strain that was resistant to colistin (MIC; 12 µg/mL), b) Another P.aeruginosa isolate resistant to colistin (MIC; 24 µg/mL)

Figure 4. Agarose gel electrophoresis of PCR products after amplification of *mcr-1* gene. MWM-molecular weight marker (50 bp DNA ladder), P1, P2, P3, P4; *P. aeruginosa* positive for *mcr-1 gene* products (320 bp).

Figure 5. Agarose gel electrophoresis of PCRproducts after amplification of *mcr-1* gene. MWM- molecular weight marker (50 bp DNA ladder), P1, P2, P3, P4; *P.aeruginosa* positive for *mcr-2 gene* products (715 bp).

Results

The study was conducted at the Medical Microbiology and Immunology Department and the central research laboratory, Faculty of Medicine, Sohag University in the period between May 2020 and May 2022. Total number of the samples collected during the study period was 225 samples isolated from patients with different types of health care-associated infections. *P. aeruginosa* strains were isolated from 75 samples (33.3%). Twelve *P. aeruginosa* strains were isolated from patients admitted at chest department (16%), 27 strains were isolated from ICU (36%), 18 strains were isolated from General surgery department (24%), 9 strains were isolated from Vascular surgery department (12%), and 9 strains were isolated from Plastic surgery (12%).

According to type of infection, *P. aeruginosa* isolation was distributed as follows: (24%) of isolates were from patients with Surgical site infection , (16%) from patients with UTI, equal percentages (12%) from patients with diabetic foot, burn infection and pneumonia, equal percentages (8%) from patients with chronic obstructive lung disease (COPD), cystic fibrosis and VAP. The highest percentage of isolation was from patients with SSI followed by UTI **(Table2)**.

Isolation of *P. aeruginosa* was higher in patients with certain risk factors such as chronic debilitating disease, patients on ventilator, prolonged hospital stay and patients with invasive devices such as IV catheters and urinary catheters with significant difference between cases and controls (*p*-value <0.05). **Table (3)**

Antibiotic susceptibility profile of *P. aeruginosa* isolates:

The antibiotic susceptibility profile of the isolated *P. aeruginosa* strains was as follows: (28%) were susceptible to piperacillin, , (12%) to ceftazidime, (12%) to cefepime, (24%) to aztreonam, (60%) to imipenem, (64%) to meropenem, (76%) to colistin, (56%) to polymixin b, (52%) to gentamicin, (40%) to tobramycin, (40%) to amikacin, (32%) to netilmycin, (36%) to ciprofloxacin, (36%) to levofloxacin, (24%) to lomefloxacin, (36%) to norfloxacin, (28%) to ofloxacin and (24%) to gatifloxacin. the highest resistance rate was to ceftazidime and cefepime followed by gatifloxacin, while the highest sensitivity was to colistin followed by meropenem **(Table 4)**.

Colistin-resistant isolates exhibited the highest resistance to polymyxin B, norfloxacin, ofloxacin and gatifloxacin by a percentage of (100%) followed by ceftazidime, cefepime, netilmycin and lomefloxacin by a percentage of (83.33%) **(Table 5)**.

The highest percentage of colistin resistant strains were isolated from patients admitted to General Surgery department that was (50%), while the other (50%) was equally distributed among patients admitted to ICU, Chest and Plastic surgery departments by a percentage of (16.67%) for each. No colistin resistant strains were isolated from

patients admitted to Vascular Surgery department. **(Table 6).**

The highest colistin-resistance rate was in isolates from SSI (50%) infection followed by isolates from cystic fibrosis patients, UTI and infected burn (16.67%). Isolates from patients with Diabetic foot, COPD, VAP, and pneumonia were colistin-sensitive (**Table 7**).

Detection of plasmid *mcr* genes among colistin resistant *P. aeruginosa* strains:

Plasmid gene *(mcr-1)* was detected in (44.4%) of colistin-resistant strains while only (16.67%) of colistin-resistant strains were positive for *(mcr-2)* gene by simple qualitative PCR (**Table 8**).

Sensitivity and specificity of E-test in detection of colistin-resistace.

The number of *P. aeruginosa* isolates that were resistant to colistin by E- test MIC measurement and were positive for any of *mcr* genes by PCR were 9 (50.0%), while isolates that were colistin-resistant according to the results of E-test and negative for any of *mcr* genes by PCR were also **9** (50.0%). No colistin susceptible isolates by E-test were positive for detection of *mcr* genes. Sensitivity of E-test in comparison with PCR was (100%), specificity was (86.36%), positive predictive value (PPV) was (50.00%), and negative predicative value (NPV) was (100%) (**Table 9**).

Table 1. Demographic data and department of admission of the study groups.

Variable	**Cases** N=75	**Controls** N=150	***p* value**
Age/year			
Mean ± SD	45.14±23.36	47.26±21.34	0.51
Median (range)	49 (0.58:77)	51 (0.58:84)	
Gender			
Female	39 (52.00%)	59 (39.33%)	0.07
Male	36 (48.00%)	91 (60.67%)	
Department			0.30
Chest	12 (16.0%)	37 (24.6%)	
ICU	27 (36.0%)	37 (24.6%)	
General surgery	18 (24.00%)	45 (30.00%)	
Vascular surgery	9 (12.00%)	16 (10.67%)	
Plastic surgery	9 (12.00%)	15 (10.00%)	

Table 2. Distribution of isolates among types of HAIs.

Variable	Cases N=75	Controls N=150	*p* value
Diabetic foot	9 (12.00%)	16 (10.67%)	0.79
Infection in COPD	6 (8.00%)	18 (12.00%)	
Infection in cystic fibrosis	6 (8.00%)	15 (10.00%)	
UTI	12 (16.00%)	22 (14.67%)	
VAP	6 (8.00%)	9 (6.00%)	
Burn wound infection	9 (12.00%)	15 (10.00%)	
Pneumonia	9 (12.00%)	10 (6.67%)	
Surgical site infection	18 (24.00%)	45 (30.00%)	

Table 3. Risk factors associated with HAIs and relation to *P. aeruginosa*- isolation.

Variable	Cases N=75	Controls N=150	*p* value
DM			
No	36 (48.00%)	65 (43.33%)	0.51
Yes	39 (52.00%)	85 (56.67%)	
Chronic debilitating disease			
No	21 (28.00%)	75 (50.00%)	0.002*
Yes	54 (72.00%)	75 (50.00%)	
Anemia			
No	18 (24.00%)	51 (34.00%)	0.13
Yes	57 (76.00%)	99 (66.00%)	
Use of broad-spectrum antibiotics			
No	27 (36.00%)	70 (46.67%)	0.13
Yes	48 (64.00%)	80 (53.33%)	
Ventilator			
No	60 (80.00%)	135 (90.00%)	0.04*
Yes	15 (20.00%)	15 (10.00%)	
Infected tracheostomy wound			
No	63 (84.00%)	131 (87.33%)	0.49
Yes	12 (16.00%)	19 (12.67%)	
Surgical drain			
No	63 (84.00%)	130 (86.67%)	0.59
Yes	12 (16.00%)	20 (13.33%)	
Urinary catheter			
No	42 (56.00%)	106 (70.67%)	0.03*
Yes	33 (44.00%)	44 (29.33%)	
IV catheter			
No	6 (8.00%)	68 (45.33%)	<0.0001*
Yes	69 (92.00%)	82 (54.67%)	
Prolonged hospital stays			
No	24 (32.00%)	80 (53.33%)	0.002*
Yes	51 (68.00%)	70 (46.67%)	
Surgical procedure			
No	48 (64.00%)	90 (60.00%)	
Minor	12 (16.00%)	28 (18.67%)	0.83
Major	15 (20.00%)	32 (21.33%)	

Table 4. Antimicrobial susceptibility profile of *P. aeruginosa* isolates.

Antibiotic	**Sensitive No. (%)**	**Intermediate No. (%)**	**Resistant No. (%)**
Piperacillin	21 (28.00%)	15 (20.00%)	39 (52.00%)
Ceftazidime	9 (12.00%)	3 (4.00%)	63 (84.00%)
Cefepime	9 (12.00%)	3 (4.00%)	63 (84.00%)
Aztreonam	18 (24.00%)	9 (12.00%)	48 (64.00%)
Imipenem	45 (60.00%)	0	30 (40.00%)
Meropenem	48 (64.00%)	0	27 (36.00%)
Colistin sulphate	57 (76%)	0	18 (24%)
Polymyxin B	42 (56.00%)	0	33 (44.00%)
Gentamycin	39 (52.00%)	0	36 (48.00%)
Tobramycin	30 (40.00%)	0	45 (60.00%)
Amikacin	30 (40.00%)	3 (4.00%)	42 (56.00%)
Netilmycin	24 (32.00%)	3 (4.00%)	48 (64.00%)
Ciprofloxacin	27 (36.00%)	0	48 (64.00%)
Levofloxacin	27 (36.00%)	6 (8.00%)	42 (56.00%)
Lomefloxacin	18 (24.00%)	9 (12.00%)	48 (64.00%)
Norfloxacin	27 (36.00%)	0	48 (64.00%)
Ofloxacin	21 (28.00%)	0	54 (72.00%)
Gatifloxacin	18 (24.00%)	0	57 (76.00%)

Table 5. antibiotic susceptibility profile of colistin-sensitive versus colistin-resistant isolates

Variable	Colistin sensitive N=57	Colistin resistant N=18	*p* value
Piperacillin	30 (52.63%)	9 (50.00%)	0.85
Ceftazidime	48 (84.21%)	15 (83.33%)	1.00
Cefepime	48 (84.21%)	15 (83.33%)	1.00
Aztreonam	39 (68.42%)	9 (50.00%)	0.16
Imipenem	21 (36.84%)	9 (50.00%)	0.32
Meropenem	18 (31.58%)	9 (50.00%)	0.16
Polymyxin B	15 (26.32%)	18 (100%)	**<0.0001***
Gentamycin	24 (42.11%)	12 (66.67%)	0.07
Tobramycin	33 (57.89%)	12 (66.67%)	0.51
Amikacin	33 (57.89%)	9 (50.00%)	0.56
Netilmycin	33 (57.89%)	15 (83.33%)	0.05
Ciprofloxacin	36 (63.16%)	12 (66.67%)	0.79
Levofloxacin	36 (63.16%)	6 (33.33%)	**0.03***
Lomefloxacin	33 (57.89%)	15 (83.33%)	0.05
Norfloxacin	30 (52.63%)	18 (100%)	**<0.0001***
Ofloxacin	36 (63.16%)	18 (100%)	**0.002***
Gatifloxacin	39 (68.42%)	18 (100%)	**0.004***

Table 6. Distribution of colistin resistant and colistin sensitive strains among different departments

Variable	Colistin Sensitive N=57	Colistin resistant N=18	*p* value
ICU	24 (42.11%)	3 (16.67%)	
Chest	9 (15.79%)	3 (16.67%)	
General surgery	9 (15.79%)	9 (50.00%)	0.02
Vascular surgery	9 (15.79%)	0	
Plastic surgery	6 (10.53%)	3 (16.67%)	

Table 7. Distribution of Colistin-sensitive and Colistin-resistant strains isolated from different types of HAIs

Variable	Colistin Sensitive N=57	Colistin resistant N=18	*p* value
Diabetic foot	9 (15.79%)	0	0.009
Infection in COPD	6 (10.53%)	0	
Infection in cystic fibrosis	3 (5.26%)	3 (16.67%)	
UTIs	9 (15.79%)	3 (16.67%)	
VAP	6 (10.53%)	0	
Burn wound infection	6 (10.53%)	3 (16.67%)	
Pneumonia	9 (15.79%)	0	
Surgical wound infection	9 (15.79%)	9 (50.00%)	

Table 8. Distribution of *mcr1* and *mcr2* genes in colistin-resistant *P. aeruginosa* isolates.

Gene	Positive		Negative	
	NO.	**%**	**NO.**	**%**
mcr1	10	55.56%	3	16.6
mcr2	8	44.4%	15	83.3

Table 9. Comparison between E- test and molecular method (PCR) regarding the detection of colistin resistance rate

	Colistin-resistant strains by E-test	**Colistin-susceptible strains by E-test**
Positive detection of *mcr* genes by PCR	True positive N = 9	False negative N = zero
Negative detection of *mcr* genes by PCR	False positive N = 9	True negative N = 57
Sensitivity (100%)		
Specificity (86.36%)		
Positive predictive value (PPV) = (50.00%)		
Negative predicative value (NPV) = (100%)		

Discussion

In this study 225 samples were collected from patients with different types of HAIs admitted at different departments at Sohag University Hospitals. *Pseudomonas aeruginosa* was isolated from 75 samples (33.3%). Phenotypically identified isolates were further confirmed by the presence of *toxA* gene (unique for *P. aeruginosa* spp.) using PCR. Sixteen percent of *P. aeruginosa* strains were isolated from patients admitted at chest department representing, (36%) were isolated from ICU, (24%) were isolated from General surgery department, (12%) were isolated from vascular surgery department, and also (12%) were isolated from Plastic surgery department**.** This means that in our study the majority of pseudomonas strains were isolated from ICU patients.

Twenty four percent of *P. aeruginosa* strains were isolated from SSIs, (16%) from patients with UTI, equal percentages (12%) from patients with diabetic foot, burn wound infection and pneumonia, and also equal percentages (8%) from patients with COPD, cystic fibrosis and ventilator associated pneumonia (VAP). The highest isolation rate of *P. aeruginosa* was from patients with SSIs followed by UTI. This distribution is different from that of study of **Marko et al.** [2] at which *P. aeruginosa* strains were isolated mainly from patients with pneumonia (50.72%), followed by SSIs (24.64%), UTI (17.91%), and bloodstream infection (BSIs) (2.72%), while other localizations were found in only 4.01%.

In our study; *P. aeruginosa* infection increases with the use of externally-associated prosthesis like ventilators (*p* value 0.04), IV catheter (P-value <0.0001), urinary catheter (*p*-value 0.03). The infection rate also increases with Prolonged hospital stay (≥33 days) (*p*-value 0.002).

Regarding the antibiotic-resistance pattern of *P. aeruginasa* isolates, resistance to piperacillin was (52%) which was slightly lower than detected in a study of **Pokharel et al**.[14], while aztreonam-resistance was (64%) which was higher than that of the study of **Farajzadeh et al.** Resistance to both ceftazidime and cefepime were **(84%)** higher than that of the same study of **Farajzadeh et al.** [15].

Resistance to imipenem (40%) and to meropenem (36%) were slightly higher than detected in the study of **Pokharel et al.** [14]. Aminoglycosides-resistance was as follows; gentamycin (48%), tobramycin (60%), amikacin (56%), netilmycin (64%), it was higher than aminoglycosides resistance in the study of **Pokharel et al.** [14]. for fluoroqquinolones; resistance to ciprofloxacine (64%), levofloxacin (56%), lomefloxacin (64%) norfloxacin (64%), ofloxacin (72%) and to gatifloxacin (76%) which was lower than **Farajzadeh et al.** [15].

According to **Pokharel et al**. [14]; resistance to piperacillin was (56.5%), to imipenem and meropenem were equally 34.7%, to gentamycin, tobramycin and amikacin were (42.2%), (28.2%) and (26%) respectively, no colistin or polymyxin B resistance detected by this study. According to **Farajzadeh et al**.[15], resistance to aztreonam was (43.7%), to ceftazidime, cefepime and ciprofloxacin was (76.7%), (80.9%) and (84.7%) respectively. Possible reasons for this variation in antibiotic resistance rates in different studies could be attributed to the difference in the local anti-biogram of different health care settings and also the genetic variability between pseudomonas isolates worldwide.

According to **Magiorakos et al.** [16], *P. aeruginosa* is defined as multidrug-resistant (MDR) if it was resistant to ≥1 agent in ≥3 antimicrobial categories, while extensively drug-resistant (XDR) if it was resistant to ≥1 agent in all but ≤2 of antimicrobial groups [16]. In our study there was a high prevalence of MDR *P. aeruginosa* (92%) of total isolates and (56%) of isolated were XDR by definition. These results were much higher than that of the study of **Tahmasebi et al.** [17], in which MDR and XDR strains were counted as 38.61% and 7.92% of isolates, respectively. These higher resistance rates could be attributed to the antibiotics over- and mis-use in our healthcare settings and also to the horizontal spread of MDR and XDR strains due to poor appliance of proper relevant infection control guidelines.

In our study, colistin resistance rate was (24%) according to the results of disc diffusion method and E-test. This result was higher than that of the study of **Tahmasebi et al.** [17] at which colistin resistance rate was (3.96%) and slightly higher than that of the study of **Abd El-Baky et al.** [18] at which colistin resistance rate was (21.3%). Those differences could also be attributed to the previously mentioned reasons. In addition, the overuse of the valuable antibiotics that are used as a last treatment option as colistin.

On re-assessment of the antibiotic susceptibility profile of colistin-resistant isolates it was found that; 50% of isolates were resistant to piperacillin which is higher than the results of **Azimi Azimi and Lari** [8]. Resistance to aztreonam was (50%), to cefepime was (83.33%), lower than that of **Azimi and Lari** [8]. Resistance to imipenem was (50%) which is much higher than that of **Azimi and Lari** [8]. Resistance to ceftazidime (83.33%), to meropenem (50%) was similar to that of **Azimi and Lari** [8]. Resistance to gentamicin and to tobramycin were the same (66.67%), to amikacin (50%) which were lower than that of **Azimi and Lari**. [8], while resistance to ciprofloxacin (66.67%) and to levofloxacin (33.33%) were much higher than that of **Azimi and Lari.** [8], Resistance to lomefloxacin was (83.33%), resistance to norfloxacin, ofloxacin and gatifloxacin were equally (100%). resistance to polymyxin B was (100%).

According to **Azimi and Lari;** (16%) of colistin resistant strains were resistant to piperacillin, (100%) to aztreonam, (84%), (96%) to ceftazidime, cefepime respectively. resistance to imipenem and meropenem were (28%) and (48%) respectively. resistance to gentamicin, tobramycin and amikacin were (92%), (84%) and (60%) respectively. resistance to ciprofloxacin and levofloxacin were equally (4%). This much higher resistance in our isolated to different antibiotic groups is accordance with the plasmid- mediated origin of resistance, as it is definitely proved that plasmids usually carry genes for multi-drug resistance.

In our study, the highest rate of colistin resistance (50%) was in strains isolated from patients with SSI followed by strains isolated from patients with cystic fibrosis, UTI and infected burn wounds (16.67%). No colistin resistant strains isolated from patients with diabetic foot, COPD, VAP and pneumonia. There was a highly significant difference between the rate of colistin resistance in strains isolated from patients with certain risk factors and patients without *(p-value* <0.05) such as: presence of chronic debilitating diseases, anemia, use of broad spectrum antibiotics, ventilator, prolonged hospital stay and urgent surgical intervention.

In this study we used simple qualitative PCR for detection of *mcr-1 and mcr-2* genes in *P. aeruginosa* isolates that were resistant to colistin. *mcr-1* gene was found in (44.44%) of isolates which was slightly lower than the result of the study of **Abd El-Baky et al.** [18] in which mcr-1 gene was found in (50%). The percentage of *mcr-2* gene detection in colistin resistant isolates was (16.67%), this result was different from that of the study of **Abd El-Baky et al.**[18] at which *mcr-2* gene was not detected at all. The change in the prevalence of resistance genes may be due to changes in antibiotic policy, the introduction and consequent inter-hospital spread of resistant strains, or the possibility that these resistance genes could be originated from an environmental source.

The emergence of colistin resistance in *P. aeruginosa* in our health care setting is an alarming issue that needs strict adherence to the infection control guidelines specially plasmid mediated resistance as it usually associated with MDR and XDR patterns.

Conflict of interest

None to be declared.

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