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ORIGINAL ARTICLE

Heteroresistance Screening of *Pseudomonas aeruginosa* Specimens from Hospitalized Inpatients in Cairo, Egypt

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ABSTRACT

Background: *Heteroresistance described infections with bacterial strains which causes infections with different levels of resistance to an antibiotic and differs with age and gender.* ***Objectives:*** *This work determined the variations in the heteroresistance pattern for the age, gender and site of infection and the statistical analysis was done using (SPSS version 20.0) for test of significance.* ***Methodology:*** *Out of two hundred and fifty clinical specimens isolated from different sites from Inpatients admitted to Kasr Al-Aini hospital and Al-Demerdash hospital from different genders with different ages in the period from February 2016 to December 2017, Egypt, one hundred and forty five specimens revealed P.aeruginosa after passing several conventional microbiological methods, the antimicrobial susceptibility testing and the screening of nht4heteroresistant specimens were performed.* ***Results:*** *The obtained results showed that the hetesroresistance was very high in the urine specimens and very high in males more than females.* ***Conclusion:*** *From the statistical analysis we recommend the use of imipenem, levofloxacin and ciprofloxacin in the treatment of heteroresistant P.aeruginosa, since they were the highly significant, effective antibiotics.*

INTRODUCTION

P.aeruginosa is a highly resistant bacteria to a wide range of antibiotics due to its ability to develop multidrug resistance and mutational acquired resistance to antibiotics [1]. It is characterized by a high intrinsic resistance to different types of antimicrobial agents and it has the capability to create resistance by mutation or acquisition of foreign resistance genes towards different classes of antibiotics[2]. *P.aeruginosa* resistance to carbapenem is very well known worldwide [3]. Carbapenem resistance may be developed by the shortage of OprD channel, the creation of different kinds of carbapenemases, including serine β-lactamases of Ambler classes A and D and metallo-β-lactamases (MBLs) of Ambler class B[4] and it causes severe problems mainly in the heteroresistant isolates[5].

The emergence of heteroresistance requires a lot of concerns and attention, and it was documented to be extremely affected by several conditions as unstable nature of the resistance phenotype, different epidemiology methods, and different conditions of the tests[6]. In the in *vitro* susceptibility testing, heteroresistance was defined as when a subset of the microbial population was resistant to an antibiotic and the other subset of the microbial population was susceptible [7]. It was clarified that the bacterial culture contains subpopulations with different categories of resistance but, the whole population is either sensitive or resistant to the antibiotic[8]. Many reports, had defined this phenomenon in the past regardless the antibiotic gradient ranges criteria[9]. In medical laboratories such heteroresistance is ignored, missed or not detected under all conditions [10]. Such type of resistance might be due to heterogeneity of plasmid or different gene expression patterns with an isogenic population [11].

P.aeruginosa outer membrane is an important barrier of the antibiotics which has low permeability, excluding the larger molecules[12]. The inability of antibiotics to accumulate in *P.aeruginosa* is due to the efflux pumps, that result in restricted permeability of the outer membrane and the efficient removal of antibiotic molecules [13].

Mutation can cause lack of OprD in *P.aeruginosa in* relatively high frequency (10^{-7}), and can be due to deletion, substitution or insertions that result in an inactivation of the *oprD*[14]. Resistance particularly results from drug inactivation by plasmid or chromosome encoded enzymes, although enzyme independent resistance from defect in uptake and accumulation also occur, mainly in CF patients and in intensive care units[15]. The main causes of *P. aeruginosa* resistance were the production of MBLs, it is characterized by its carbapenemase activity, quick distribution, resistance to β-lactamase inhibitors and the hydrolysis of all β-lactam antibiotics[16].

P.aeruginosa colonises human body sites, mainly the moist areas, such as the ear, nasal mucosa and

throat, as well, as stools and is present also on some plants, the colonization is very rare in normal persons, it occurs in high rate in hospitalzed patients mainly after a long-term use of broad [17]. Therefore, *P.aeruginosa* is mostly a nosocomial pathogen. It causes nosocomial respiratory tract infections including ventilator-associated pneumonia (VAP), dermatitis, soft tissue infections, bacteraemia, bone and joint infections, gastrointestinal infections and a variety of systemic infections, particularly in immunosuppressed patients (AIDS), or patients with severe burns or cancer [18] .

Heteroresistance was reported to be highly affected by many variables such as the screening procedures, local epidemiology methods, different conditions of the test, unstable nature of the resistance phenotype and antibiotic selective pressure among the different pathogen strains and antimicrobials [19].

METHODOLOGY

Bacterial isolates:

Out of two hundred and fifty clinical specimens, that were submitted for bacteriological testing from hospitalized inpatients from different clinical sources, genders and different ages admitted to Kasr Al Aini Hospital and Al-Demerdash Hospital, Cairo, Egypt in the period from February 2016 till December 2017, one hundred and forty five samples revealed *Pseudomonas aeruginosa.*

This research was approved according to the criteria of the Egyptian Network of the Research Ethics committee.

The one hundred and forty five *P.aeruginosa* isolates were identified using the conventional methods done by Monica Cheesbrough on the basis of Gram staining, motility, pigment production, catalase test, and specific colony morphologies [20].

Antimicrobial Susceptibility Testing (Disk Diffusion Method):

The following antibiotics were used, ampicillin 10µg (AMP^{10}), ampicillin/sulbactam 20µg (SAM^{20}), trimethoprim/sulphamethoxazole 25µg (SXT^{25}), imipenem 10 µg (IPM^{10}), ciprofloxacin 5 µg (CIP^{5}), gentamicin 10 µg (CN^{10}), levofloxacin 5 µg (LEV^{5}), polymyxin B 300 U (PB^{300}), ceftriaxone 30 µg (CRO^{30}) and tetracycline TE 30 µg (TE^{30}), supplied from (Himedia-India) in the antimicrobial susceptibility testing according to Bauer and Kirby method [21] and then screening of the heteroresistant strains of resistant *P.aeruginosa* was recorded.

Statistical Analysis:

Data generated in this study was analyzed using statistical software (SPSS version 20.0) for test of significance. Results were presented as percentages. Relationships at a p-value of less than or equal to 0.05 ($P \leq 0.05$) was considered statistically significant. The heteroresistance distribution was determined according to the site, age and gender.

RESULTS

Identification of *Pseudomonas aeruginosa*:

The one hundred forty five *P.aeruginosa* isolates had shown positive morphological characters on MacConkey's agar, Gram stain, motility, pigment production, catalase test and oxidase test.

Antimicrobial Susceptibility Testing using KB method and detection of the heteroresistant isolates:

The appearance of heteroresistance was demonstrated in the figure.1

Fig.1. Heteroresistance pattern of imipinem antibiotic

Screening of heteroresistance in resistant *P.aeruginosa* clinical isolates had reported the detection of forty three heteroresistant isolates and they are represented in table 1 with the age, site, and gender.

Table 1: Heteroresistance pattern according to the site of infection

Isolates Number	Isolate Site	Heteroresistance detected in the following antibiotics
P3	Urine	Ampicillin, Ceftriaxone, Tetracyclin and Polymixin B.
P11	Ear Wash	Ampicillin/Sulbactam, Imipinem and polymyxin B
P15	Urine	Imipinem, Ampicillin and Tetracyclin.
P16	CSF	Gentamicin, Sulfamethoxazole/Trimethoprim and Ampicillin.
P18	Blood	Ampicillin/Sulbactam, Tetracyclin and Polymixin B.
P21	Ear wash	Ceftriaxone, Ampicillin/Sulbactam, Ampicillin and Ciprofloxacin.
P24	CSF	Ceftriaxone, Ampicillin/Sulbactam and Tetracyclin.
P27	CSF	Gentamicin, Ampicillin and Polymixin B.
P28	Urine	Ceftriaxone, Sulfamethoxazole/Trimethoprim and Ampicillin.
P30	Urine	Ampicillin, Tetracyclin and Polymixin B.
P32	Urine	Ampicillin, Ciprofloxacin and Ceftriaxone.
P34	Urine	Ceftriaxone, Tetracyclin and Ampicillin.
P43	Urine	Ampicillin/Sulbactam, Ampicillin and Polymixin B.
P46	Wound	Ampicillin and Polymixin B.
P50	Ear wash	Ampicillin, Ceftriaxone and Levofloxacin.
P51	Urine	Ceftriaxone, Ampicillin/Sulbactam, Ampicillin and Ciprofloxacin.
P52	Urine	Ampicillin/Sulbactam, Ampicillin and Tetracyclin.
P61	Urine	Ampicillin/Sulbactam, Ampicillin and Tetracyclin.
P64	Wound	Ampicillin and Polymixin B.
P66	Urine	Ampicillin, Ciprofloxacin and Polymixin B.
P67	Blood	Ampicillin and Tetracyclin.
P74	Urine	Ceftriaxone, Ampicillin/Sulbactam and Ampicillin.
P75	Blood	Ampicillin/Sulbactam, Ampicillin and Polymixin B.
P78	Sputum	Ampicillin, Tetracyclin and Polymixin B.
P80	Blood	Ceftriaxone and Ampicillin.
P83	CSF	Ceftriaxone, Ampicillin/Sulbactam and Ampicillin.
P87	Blood	Ampicillin/Sulbactam and Ciprofloxacin.
P89	Ear wash	Ampicillin and Tetracyclin.
P91	Sputum	Ceftriaxone, Ampicillin and Ciprofloxacin.
P94	Urine	Ampicillin, Tetracyclin and Sulfamethoxazole / Trimethoprim
P96	Urine	Ampicillin, Ciprofloxacin and Ceftriaxone.
P97	Ear wash	Ampicillin and Polymixin B.
P105	Urine	Ampicillin/Sulbactam and Tetracyclin.
P107	Urine	Ampicillin, Ciprofloxacin and Polymixin B.
P116	Urine	Ampicillin and Polymixin B.
P118	Urine	Ampicillin/Sulbactam, Ceftriaxone and Tetracyclin.
P122	Urine	Ampicillin and Polymixin B.
P125	Urine	Ceftriaxone and Ampicillin.
P128	CSF	Ceftriaxone, Sulfamethoxazole / Trimethoprim, Ampicillin and Gentamicin.
P133	Ear wash	Ampicillin, Tetracyclin and Polymixin B.
P138	Blood	Ceftriaxone, Ampicillin and Polymixin B.
P141	CSF	Ampicillin and Tetracyclin.
P145	CSF	Ampicillin/Sulbactam, Ceftriaxone and Tetracyclin.

From the previous results, we recorded that the heteroresistant samples in the urine were 20 samples with 46.5%. In the blood and ear washes, 6 samples each with the percentage 13.96%. In the cerebrospinal fluid, the total heteroresistant samples were 7(16.28%). The wound and blood heteroresistant specimens were 2 each with 4.65% ratio.

Table 2: Heteroresistance pattern according to the age and gender of cases

Source	Number of Samples	Age range	Gender
Blood	6	23-65 years	4 Male 2 Female
Cereprospinal fluid	7	23-81 years	6 Male 1 Female
Ear washes	6	13-59 years	3 Male 3 Female
Sputum	2	5-63 years	0 Male 2 Female
Urine	20	2-76 years	13 Male 7 Female
Wound	2	10-71 years	2 Male 0 Female

We have reported that, the distribution of hetroresistance was 28 isolates in the males with the ratio 65.12% and 15 in females with the ratio 34.49%. The heteroresistance distribution were high in the age above 63 years and low in the age less than 23 years.

Statistical Analysis Results:

The distribution of Heteroresistance according to different age groups is shown in table 3.

Table 3: Heteroresistance distribution pattern in different age groups

Age		Mean	S.D	N of total heteroresistant isolates
Ceftriaxone	Teen (<25)	6.45	9.634	11
	Adult (25-50)	8.50	9.499	14
	Old (>50)	19.75	17.123	18
	Total	12.69	14.356	43
Ampicillin_Sulbactam	Teen (<25)	41.09	19.927	11
	Adult (25-50)	38.57	21.717	14
	Old (>50)	42.00	19.415	18
	Total	40.65	19.884	43
Gentamicin	Teen (<25)	4.82	9.247	11
	Adult (25-50)	1.52	.973	14
	Old (>50)	8.36	11.514	18
	Total	5.23	9.119	43
Tetracyclin	Teen (<25)	35.09	24.321	11
	Adult (25-50)	27.50	18.105	14
	Old (>50)	30.33	17.918	18
	Total	30.63	19.513	43
Trimethoprim_ Sulfamethoxazole	Teen (<25)	52.36	16.145	11
	Adult (25-50)	56.00	16.305	14
	Old (>50)	58.67	12.271	18
	Total	56.19	14.552	43
Imipenem	Teen (<25)	3.70	9.396	11
	Adult (25-50)	.80	.612	14
	Old (>50)	1.69	3.636	18
	Total	1.92	5.269	43
Ampicillin	Teen (<25)	49.45	16.711	11
	Adult (25-50)	41.14	15.002	14
	Old (>50)	40.89	14.748	18
	Total	43.16	15.432	43
Ciprofloxacin	Teen (<25)	3.65	5.070	11
	Adult (25-50)	2.81	4.635	14
	Old (>50)	3.79	8.071	18
	Total	3.44	6.272	43
Levofloxacin	Teen (<25)	3.85	6.102	11
	Adult (25-50)	1.13	1.072	14
	Old (>50)	3.46	8.010	18
	Total	2.80	6.050	43
Polymixin_B	Teen (<25)	13.50	13.090	11
	Adult (25-50)	20.75	19.831	14
	Old (>50)	15.74	14.958	18
	Total	16.80	16.175	43

Table 4: Antibiotics efficacy according the significance (P≤ 0.05) for the age group ranges from 23 to 63 years old.

antibiotics	Significance
Gentamicin	.002
Ceftriaxone	.008
Imipenem	.034
Levofloxacin	.056
Trimethoprim_Sulfamethoxazole	.147
Tetracyclin	.152
Ampicillin	.344
Polymixin_B	.495
Ciprofloxacin	.611
Ampicillin_Sulbactam	.917

Table 4 showed, Imipinem, Ceftriaxone and Gentamicin were the most effective antibiotics for the age group ranges from 23 to 63 years old.

Table 5: Heteroresistance distribution pattern according to gender

Gender		Mean	S.D	N of total heteroresistant isolates
Ceftriaxone	Male	14.54	15.415	27
	Female	9.56	12.191	16
	Total	12.69	14.356	43
Ampicillin_Sulbactam	Male	40.59	19.929	27
	Female	40.75	20.460	16
	Total	40.65	19.884	43
Gentamicin	Male	6.16	9.918	27
	Female	3.66	7.626	16
	Total	5.23	9.119	43
Tetracyclin	Male	30.93	20.128	27
	Female	30.13	19.064	16
	Total	30.63	19.513	43
Trimethoprim_ Sulfamethoxazole	Male	55.11	15.584	27
	Female	58.00	12.900	16
	Total	56.19	14.552	43
Imipenem	Male	1.52	2.950	27
	Female	2.59	7.867	16
	Total	1.92	5.269	43
Ampicillin	Male	42.67	15.372	27
	Female	44.00	16.000	16
	Total	43.16	15.432	43
Ciprofloxacin	Male	3.39	5.272	27
	Female	3.52	7.871	16
	Total	3.44	6.272	43
Levofloxacin	Male	3.32	7.044	27
	Female	1.92	3.895	16
	Total	2.80	6.050	43
Polymixin_B	Male	16.89	16.743	27
	Female	16.64	15.705	16
	Total	16.80	16.175	43

Table 6: Antibiotics efficacy according the significance (P≤ 0.05) for the gender

Antibiotics	Significance
Imipenem	.107
Gentamicin	.142
Trimethoprim_Sulfamethoxazole	.182
Levofloxacin	.194
Ceftriaxone	.471
Ampicillin	.606
Polymixin_B	.657
Tetracyclin	.742
Ciprofloxacin	.960
Ampicillin_Sulbactam	.974

Table 6 reported that, Levofloxacin, Trimethoprim/sulfamethoxazole, Gentamicin and Imipinem were the most effective antibiotics for males and females.

Table 7: Heteroresistance distribution pattern according to the site of infection

Source		Mean	S.D	N of total heteroresistant isolates
Ceftriaxone	Urine	8.68	9.892	20
	Ear wash	9.00	12.681	6
	CSF	30.29	18.455	7
	Blood	11.00	11.696	6
	Wound	2.00	0.000	2
	Sputum	18.00	19.799	2
	Total	12.69	14.356	43
Ampicillin_Sulbactam	Urine	44.00	17.119	20
	Ear wash	38.00	22.874	6
	CSF	50.29	17.105	7
	Blood	30.67	19.211	6
	Wound	4.00	0.000	2
	Sputum	48.00	22.627	2
	Total	40.65	19.884	43
Gentamicin	Urine	2.23	2.209	20
	Ear wash	8.00	12.000	6
	CSF	17.00	14.866	7
	Blood	1.29	.813	6
	Wound	1.25	1.061	2
	Sputum	1.50	.707	2
	Total	5.23	9.119	43
Tetracyclin	Urine	34.40	18.914	20
	Ear wash	32.33	26.303	6
	CSF	29.71	17.105	7
	Blood	26.67	19.377	6
	Wound	1.50	.707	2
	Sputum	32.00	0.000	2
	Total	30.63	19.513	43
Trimethoprim_Sulfamethoxazole	Urine	53.60	16.640	20
	Ear wash	58.67	13.064	6
	CSF	64.00	0.000	7
	Blood	58.67	13.064	6
	Wound	64.00	0.000	2
	Sputum	32.00	0.000	2
	Total	56.19	14.552	43
Imipenem	Urine	1.74	3.410	20
	Ear wash	5.85	12.813	6
	CSF	.86	.789	7
	Blood	.79	.710	6
	Wound	.50	0.000	2
	Sputum	.38	.177	2
	Total	1.92	5.269	43
Ampicillin	Urine	40.00	14.216	20
	Ear wash	53.33	16.525	6
	CSF	36.57	12.095	7
	Blood	53.33	16.525	6
	Wound	32.00	0.000	2
	Sputum	48.00	22.627	2
	Total	43.16	15.432	43
Ciprofloxacin	Urine	3.64	5.179	20
	Ear wash	2.50	3.000	6
	CSF	.50	.354	7
	Blood	3.33	6.210	6
	Wound	.19	.088	2
	Sputum	18.00	19.799	2
	Total	3.44	6.272	43
Levofloxacin	Urine	3.16	7.607	20
	Ear wash	6.25	7.673	6
	CSF	.54	.359	7
	Blood	.81	.688	6
	Wound	4.00	0.000	2
	Sputum	1.50	.707	2
	Total	2.80	6.050	43
Polymixin_B	Urine	18.30	18.581	20
	Ear wash	12.17	10.815	6
	CSF	16.00	8.000	7
	Blood	23.00	22.477	6
	Wound	16.00	0.000	2
	Sputum	.63	.530	2
	Total	16.80	16.175	43

Table 8: Antibiotics efficacy according the significance (P≤ 0.05) for the site of infection

Antibiotics	Significance
Gentamicin	.000
Ciprofloxacin	.000
Trimethoprim_Sulfamethoxazole	.000
Imipenem	.003
Ampicillin	.083
Levofloxacin	.093
Polymixin_B	.126
Ampicillin_Sulbactam	.131
Tetracyclin	.146
Ceftriaxone	.437

From the obtained results, Imipenem, Trimethoprim/Sulfamethoxazole, Ciprofloxacin and Gentamicin were reported to be the most effective antibiotics for the different sites of infection.

DISCUSSION

In the present study, variations in *P.aeruginosa* prevalence may be due to differences in study population, number of specimens, exposure to broad spectrum antibiotics and contact with hospital settings[22]. Heteroresistance is a special type of bacterial resistance and our study had given more concern to this kind of resistance because it can lead to false clinical detection and the therapy failure. The first study of heteroresistance was performed on *Staphylococcus aerus*[23], as first discovered on methicillin heteroresistant *Staphylococcus aerus* isolates from sputum specimens from one patient with infectious disease. Many countries until now have been reported the vancomycin heteroresistant *Staphylococcus aerus* [24].

In a study done to demonstrate the differences in distribution of *P.aeruginosa* in Australian and Indian isolates from keratitis, it was reported that it the organism is highly distributed in India more than Australia, and this is due to the hazardous use of antibiotics in India [25]. Our results agreed with this study as we also, use the antibiotics haphazardly and we found that the heteroresistant isolates were highly distributed in urine samples.

In our study, we used different clinical samples to make the statistical analysis. We noticed that, the age distribution range was very wide in the urine clinical specimens, sputum and in wound and relatively narrow in the ear wash clinical specimens.

Heteroresistance is considered to be a phenomenon in which subpopulations of a certain bacteria showed different susceptibilities to a specific antibiotic [26].

Heteroresistance acts as an intermediate stage, which is considered to change from a susceptibility to full resistance under different or sudden conditions [27].

In another study that was done in China, the study proved that while collecting the isolates from patients, who had never been treated by colistin, they observed that the resistance and heteroresistance might not be correlated to previous exposure to colistin, they reported that colistin heteroresistance may function as a resistance reservoir, thus leading to the dissimination of resistant isoaltes when exposed to colistin [28].

In the present work, heteroresistance distribution is in a high ratio in the old age people, especially those whose samples were taken from the cerebrospinal fluids. This phenomenon must be paid more concern in the upcoming years and the awareness of the people should be increased to avoid the wide spread of heteroresistance complications by the misuse of the antibiotics. Our study have also observed, the most effective antibiotics for the site of infection, age and gender were imipenem, gentamicin, ciprofloxacin and levofloxacin, and these drugs will be advised in the treatment of heteroresistant *P.aeruginosa* clinical specimens

CONCLUSIONS

Our study showed that heteroresistance distribution of *P.aeruginosa* according to the age, site and gender were determined using statistical analysis SPSS version 20.00 and imipenem, ciprofloxacin and levofloxacin would be the drugs of choice in the cure for the different ages, genders and samples.

Our study recommends further studies and researches to be done in the future to make drug combinations of the previously mentioned antibiotics to speed up the recovery and make a quick improvement for the cases.

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REFERENCES

1. Al-Grawi I. Expression of mexAB-oprM Operon of Septicemic Pseudomonas aeruginosa in relation to antibiotic resistance. Ph. D Thesis, College of Medicine, Al-Nahrain University 2011.
2. Strateva T, Yordanov D. Pseudomonas aeruginosa–a phenomenon of bacterial resistance. Journal of medical microbiology 2009; 58(9), 1133-1148.
3. Nagao M, Iinuma Y, Igawa J, Saito T, Yamashita K, Kondo T, Ichiyama S. Control of an outbreak of carbapenem-resistant Pseudomonas aeruginosa in a haemato-oncology unit. Journal of Hospital Infection 2011;79(1), 49-53.
4. Quale J, Bratu S, Gupta J, Landman D. Interplay of efflux system, ampC, and oprD expression in carbapenem resistance of Pseudomonas aeruginosa clinical isolates. Antimicrobial agents and chemotherapy 2006; 50(5), 1633-1641.
5. El-Halfawy OM, Valvano MA. Chemical communication of antibiotic resistance by a highly resistant subpopulation of bacterial cells. PloS one 2013; 8(7), e68874.
6. Plipat N, Livni G, Bertram H, Thomson RB, Jr. Unstable vancomycin heteroresistance is common among clinical isolates of methiciliin-resistant Staphylococcus aureus. J Clin Microbiol 2005; 43(5), 2494-2496. doi: 10.1128/jcm.43.5.2494-2496.2.
7. Falagas ME, Makris GC, Dimopoulos G, Matthaiou DK. Heteroresistance: a concern of increasing clinical significance? Clin Microbiol Infect 2008; 14(2), 101-104. doi: 10.1111/j.1469-0691.2007.01912.
8. Kayser FH, Benner EJ, Hoeprich PD. Acquired and native resistance of Staphylococcus aureus to cephalexin and other beta-lactam antibiotics. Appl Microbiol 1970; 20(1), 1-5.
9. Ryffel C, Strässle A, Kayser FH, Berger Bächi B. Mechanisms of heteroresistance in methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 1994;38(4), 724-728. doi: 10.1128/aac.38.4.724
10. Hopkins KL, Davies RH, Threlfall EJ. Mechanisms of quinolone resistance in Escherichia scoli and Salmonella: recent developments. International journal of antimicrobial agents 2005; 25(5), 358-373.
11. Torres OR, Korman RZ, Zahler SA, Dunny GM. The conjugative transposon Tn 925: enhancement of conjugal transfer by tetracycline in Enterococcus faecalis and mobilization of chromosomal genes in Bacillus subtilis and E. faecalis. Molecular and General Genetics MGG 1991; 225(3), 395-400.
12. Dinesh S, Grundmann H, Pitt T, Römling U. European-wide distribution of Pseudomonas aeruginosa clone C. Clinical microbiology and infection 2003; 9(12), 1228-1233.
13. Pearson JP, Van Delden C, Iglewski BH. Active efflux and diffusion are involved in transport of Pseudomonas aeruginosa cell-to-cell signals.1999; Journal of bacteriology, 181(4), 1203-1210.
14. Paterson DL. Resistance in gram-negative bacteria: Enterobacteriaceae. American journal of infection control. American journal of infection 2006;34(5).
15. Azucena E, Mobashery S. Aminoglycoside-modifying enzymes: mechanisms of catalytic processes and inhibition. Drug Resist Updat 2001;4(2),106-117. doi:10.1054/drup.2001.0197.
16. Cornaglia G, Giamarellou H, Rossolini GM. Metallo-β-lactamases: a last frontier for β-lactams? The Lancet infectious diseases 2011; 11(5), 383-393.
17. Ramos JL. Pseudomonas: Volume 1 Genomics, Life Style and Molecular Architecture: Springer Science & Business Medi 2011; 23(2).
18. Gómez Zorrilla S, Camoez M, Tubau F, Cañizares R, Periche E, Dominguez M, Peña C. Prospective observational study of prior rectal colonization status as a predictor for subsequent development of Pseudomonas aeruginosa clinical infections. Antimicrobial agents and chemotherapy 2015;59(9), 5213-5219.
19. Yamazumi T, Pfaller MA, Messer SA, Houston AK, Boyken L, Hollis RJ, Jones RN. Characterization of heteroresistance to fluconazole among clinical isolates of Cryptococcus neoformans. J Clin Microbiol 2003;41(1), 267-272. doi: 10.1128/jcm.41.1.267-272.
20. Cheesbrough M. District Laboratory Practice in Tropical CountriesPart 2 2006; from https://doi.org/10.1017/CBO9780511543470.
21. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 1966; 36:493-496.

22. Mahmoud AB., Zahran WA., Hindawi, GR, Labib, A Z, Galal, R. Prevalence of multidrug-resistant Pseudomonas aeruginosa in patients with nosocomial infections at a university hospital in Egypt, with special reference to typing methods. J Virol Microbiol 2013; 165-159.
23. Chabbert Y. Behaviour of" methicillin hetero-resistant" staphylococci to cephaloridine. Postgraduate medical journal 1976; 43, Suppl 43: 40-42.
24. Campanile F, Borbone S, Perez M, Bongiorno D, Cafiso V, Bertuccio T, Stefani S. Heteroresistance to glycopeptides in Italian meticillin-resistant Staphylococcus aureus (MRSA) isolates. International journal of antimicrobial agents 2010; 36(5), 415-419.
25. Wilhelmus KR, Abshire RL, Schlech BA. Influence of fluoroquinolone susceptibility on the therapeutic response of fluoroquinolone-treated bacterial keratitis. Arch Ophthalmol 2003; 121(9), 1229-1233. doi: 10.1001/archopht.121.9.1229.
26. Charretier Y, Diene SM, Baud D, Chatellier S, Santiago Allexant E, van Belkum A, Schrenzel J. Colistin Heteroresistance and Involvement of the PmrAB Regulatory System in Acinetobacter baumannii. Antimicrob Agents Chemother 2018; 62(9). doi: 10.1128/aac.00788-18.
27. Superti SV, Martins DdS, Caierão J, Soares FdS, Prochnow T, Zavascki AP. Indications of carbapenem resistance evolution through heteroresistance as an intermediate stage in Acinetobacter baumannii after carbapenem administration. Revista do Instituto de Medicina Tropical de São Paulo 2009; 51(2), 111-113.
28. Yau W, Owen RJ, Poudyal A, Bell JM, Turnidge JD, Heidi HY, Li J. Colistin hetero-resistance in multidrug-resistant Acinetobacter baumannii clinical isolates from the Western Pacific region in the SENTRY antimicrobial surveillance programme. Journal of Infection 2009; 58(2), 138-144.