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Research Article

COMPARISON OF CARBAPENEM RESISTANCE AMONG CLINICAL AND ENVIRONMENTAL *PSEUDOMONAS AERUGINOSA* IN SOUTH WEST NIGERIA

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ABSTRACT

Pseudomonas aeruginosa (P.A) is an opportunistic pathogen and one of the major causes of community and opportunistic infection in our environs. Infections resulting from this pathogen are very difficult to treat. The emergence of resistance caused by this organism is really alarming which have created serious health problems resulting in enormous burden of morbidity, mortality and high health care and management costs among it victims.

The aim of this research is to identify and determine prevalence of P.A, evaluate their antibiotic susceptibility patterns and compare carbapenem resistance among clinical and environmental *Pseudomonas aeruginosa* isolates from selected hospitals.

This study was conducted in four tertiary hospitals in south west Nigeria between January and December, 2016 using standard laboratory procedures. Kirby-Bauer disc diffusion method was used for susceptibility testing according to CLSI 2015.

172 clinical and 20 environmental P.A were recovered from 1338 clinical and 2230 environmental samples collected from four selected hospitals. Prevalence of 12.8% and 0.9% was found among clinical and environmental isolates respectively. Clinical P.A were 92.5% (159 of 172) cephalosporin resistant, 28.5.% (49 of 172) carbapenem resistant, while 90% cephalosporin and 10% (2 of 20) carbapenem resistant strains were detected from environmental P.A respectively. Nosocomial and community acquired infections were found to be 66.3% (114/172) and 33.7% (58/172) respectively while Pus/wound predominated as sources of the isolates, with 55.2% (95/172). Carbapenem resistance in P.A infections is increasing day by day and more stringent infection control policies should be institutionalised in our hospital settings, to stem the trend.

INTRODUCTION

Pseudomonas aeruginosa remains one of the most important pathogens responsible for nosocomial infections and it is also an opportunistic pathogens. It is a Gram negative Bacilli, motile and aerobic in nature. The pathogen contributes strongly to nosocomial infections and affect immunocompromised individual (1). Infections caused by *Pseudomonas aeruginosa* include urinary tract infection, skin and soft tissue infection, pneumonia among others. Infections caused by this organism are sometimes life threatening, severe and very difficult to treat due to its compromised susceptibility to antimicrobial agents and high frequency of emergence of antibiotics resistant strains.

Resistant to carbapenem (imipenem, meropenem, doripenem and ertapenem) among *Pseudomonas aeruginosa* is really worrisome because this class of β-lactam antibiotics represents the last therapeutic option to control infections caused by the organism. Even though efflux pumps and porins may contribute to carbapenem resistant phenotypically, production of carbapenem hydrolysing enzymes is the most relevant resistance mechanism (2). Ever since the first class metallo beta lactamase (MBLs) in *Pseudomonas aeruginosa* were identified in Japan in the year 1991 (2), MBLs have been reported for *Pseudomonas aeruginosa* isolates from nearly all regions of the world (3). Carbapenem resistant *Pseudomonas aeruginosa* is being reported commonly in some of our hospitals in Nigeria, this day (4), hence the need to know the prevalence in our

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hospital settings in order to help in the empirical treatment of patients.

The objectives of this research is therefore to identify and determine prevalence of *Pseudomonas aeruginosa*, evaluate their antibiotic susceptibility patterns and compare carbapenem resistant among clinical and environmental *Pseudomonas aeruginosa* isolates from some tertiary hospitals in south west Nigeria.

Community acquired infections in this content are infections from the communities or infections acquired within 24hrs of admission in the hospital while nosocomial infection refers to infections acquired after 24hours of admission into the hospital. Carbapenem resistance will be defined as any strain that shows resistance to at least one out of three Carbapenem antibiotics tested (5).

MATERIALS AND METHODS

The study was carried out in the Department of Medical Laboratory Sciences, Ladoke Akintola University of Technology (Mercy Land), Osogbo from January to December, 2016. A total of 1338 samples from wound swabs, urine specimen, stools, blood specimen, sputum, ear swabs and throat swabs submitted to Microbiology Departments of four tertiary hospitals in South West Nigeria namely: LAUTECH Teaching Hospital-Osogbo, LAUTECH Teaching Hospital-Ogbomoso, Federal Medical Center-Abeokuta and University College Hospital-Ibadan; and 2230 environmental samples recovered from sinks, beddings, equipment, furniture and walls of the four tertiary hospitals were processed following standard microbiology procedures. Ethical approval was gotten from the ethical committees of the four tertiary hospitals.

Identification of Pseudomonas aeruginosa

Isolation and preliminary screening of *Pseudomonas aeruginosa* was done by conventional methods. Clinical Samples from wound swab, blood, urine, sputum, ear swab, throat swab and stool were cultured by streaking on MacConkey chocolate and Sabouroud agar plates with a sterile wire loop using standard procedure. The plates were incubated aerobically overnight at 37˚C.

Hospital environment samples were also processed using conventional method. Samples from sinks, equipment, beddings were collected using a sterile swab stick soaked with peptone water and were cultured immediately using a streak method with a sterile wire loop on chocolate and MacConkey agar, then incubated overnight at 37˚C.

Isolates presumed to be *Pseudomonas aeruginosa* were identified by using colonial morphology, Gram reactions, motility test, biochemical tests, production of blue green pigment pyocyanin and also its ability to grow at 42^{O}C.

Antibiotics Susceptibility Testing

Pseudomonas aeruginosa isolates were tested for susceptibility using Kirby-Baurer techniques on Mueller Hilton (MH) agar plates (Oxiod). Sterile wire loop was used to pick test organism suspended in Mueller Hilton broth and incubated at 37 ºC for 2 hours. Turbidity of the suspension was adjusted to 0.5 Macfarland's standard (1.5x 10^8 CFU/ml) Sterile swab was then dipped into the inoculum tube and pressed against the inner side of the tube to remove excess fluids (streak plate). The entire surface of the MH agar was streaked evenly in three directions to ensure even distribution and this was allowed to stay for 3 minutes. A sterile forceps was then used to place antimicrobial disks on the plate and was pressed lightly to ensure contact with the agar. The following antibiotic discs (oxoid products)-Gentamycin (CN-10µg), Cefotaxime (CTX-5µg), Ceftazidime (CAZ-10µg), Ciprofloxacin(CIP-10µg), Streptomycin(S-10µg), Augumentin(AMC-30µg), Ofloxacin (OFX-5µg),Amoxicillin (AM-10µg),Imipenem (IPM-10µg), Meropenem (MEM-10µg) and Doripenem (10µg) were used against the isolate on MH plates incubated at 37ºC overnight. The disk were placed at a distance of 15mm from the edge of the plate and 25mm from one disk to another .it was then incubated at 37 ºC within 30 minutes of applying the disks for 18 hours aerobically. A confluent 'lawn' of growth was obtained. Controls were set along with the test.

Zone diameter of inhibition (in mm) of the organism to each antibiotic were measured with a calibrated ruler put on the underside of the plate and was interpreted as susceptible, intermediate or resistance to each antimicrobial agent tested according to CLSI (2005).

Ethical Approval: Ethical approval was gotten from the four selected hospitals.

Data analysis -Data were generated from the results and SPSS 24 was used to analyze it. Results were generated through frequency and percentage. Chi-square was used as statistical tool.

RESULTS

Out of the one thousand three hundred and thirty eight (1338) clinical samples and two thousand two hundred and thirty(2230) environmental samples collected from the four tertiary hospitals in South West Nigeria *Staphylococcus* species predominated among the clinical samples with 16.5% (221), followed by *Klebsiella* species 14.7%(197), *Pseudomonas aeruginosa* 12.8% (172). The proportions of other isolates include *Proteus* species 4.9% (65), *Escherichia coli* 3.5% (47) *Streptococcus* species 2.6% (35), Fungi 1.9% (25) *Serrentia* species 0.2% (3) while those without any growth were 42.9% (573). Among the environmental sample, *Klebsiella* species has the highest percentage of 15.7% (350), followed by *Staphylococcus* species 7.4% (165), *Escherichia coli* 3.5% (78), Fungi 2.6% (57) and *Pseudomonas species* 0.9% (20), while the proportion without *growth* was 68.1% (1520). These details are depicted in Table 1.

The one hundred and seventy two (172) *Pseudomonas aeruginosa* were then analysed for demographic profile. Gender of patients from whose samples the pathogens were isolated shows male to be 87 (50.6%) and female to be 85 (49.4%) as shown in Figure 1. Age distribution shows age range 21-30: (30)17.4%, 31-40 to be 58 (33.8%), 41-50: 38 (22%), 51 and above 24 (14%). Table 2 reflects the details of age distribution of the participants.

Sources of the isolates as reflected in sites of sample collection shows that 55.2% (95) were from pus/wound swab followed by urine; 23.3%(40), ear: 12.8% (22) and blood: 8.7%(15) Figure 2. Nosocomial infection-associated *Pseudomonas aeruginosa* was found to be 114 (65.3%) while community acquired

infection-associated *Pseudomonas aeruginosa* was 58 (34.7%); as depicted in Figure 3.

Among the clinical isolates *Pseudomonas aeruginosa* shows resistance to Cephalosporin with ceftazidime and cefotaxime constituting 92.5% and 92.1% respectively. Other antibiogram revealed: Streptomycin-141 (82%), Gentimicin-136 (79%), Amoxicillin-137 (76.6%), Augumentin-112 (68%), Ciprofloxacin-71(41.2%), Ofloxacin-76 (44.1%), Imipenem-13.9% (24), Meropenem-13.4% (23) and Doripenem (28.5%) while the 20 environmental *Pseudomonas aeruginosa* shows resistance to Cephalosporin with cefotaxime and Ceftazidine leading in high proportions (>90%). This and other details are as reflected in Table 3.

Comparison of carbapenem resistance among the clinical and environmental isolates shows that clinical *Pseudomonas aeruginosa* are more resistant to carbapenem with 28.5% (49 out of 172) while environmental *Pseudomonas aeruginosa* was 10% (2 out of 20)

Table 1 Microbial Isolates from Some Tertiary Hospitals in Southwest Nigeria

Microorganism isolated	Clinical Samples		Environmental Samples	
	Frequency	Percentage	Frequency	Percentage
Pseudomonas aeruginosa	172	12.8	20	0.9
Staphylococcus Species	221	16.5	165	7.4
Klebsiella Species	197	14.7	350	15.7
Proteus Species	65	4.9	40	1.8
Escherichia coli	47	3.5	78	3.5
Streptococcus Species	35	2.6	0	0
Fungi	25	1.9	57	2.6
Serrentia Species	3	0.2	0	0
No Growth	573	42.9	1520	68.1
Total	1338	100	2230	100

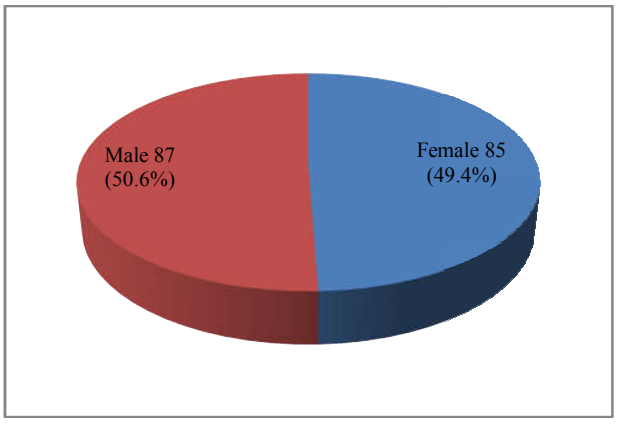

Figure 1 Gender Distribution of patients whose samples yielded growth of *Pseudomonas aeruginosa* (n=172).

Table 2 Age Distribution of Patients whose samples yielded growth of *Pseudomonas aeruginosa* (n=172)

Age Group (years)	Frequency	Percentage
0- 10	7	4.1
10 - 20	15	8.7
21 - 30	30	17.4
31 - 40	58	33.8
41 - 50	38	22
51 and Above	24	14
Total	172	100

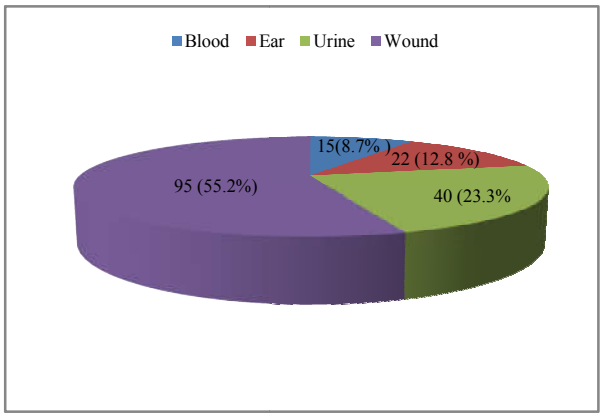

Figure 2 Distribution of *Pseudomonas aeruginosa* from Different Clinical Samples from Four Tertiary Hospital in South West Nigeria (n=172)

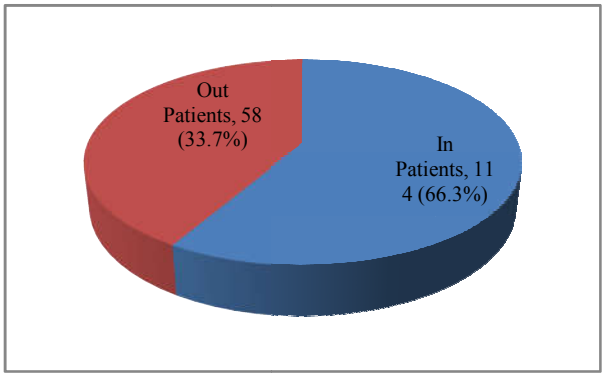

Figure 3 Distribution of *Pseudomonas aeruginosa* among In and Out Patients of the Four Tertiary Hospitals in South West Nigeria (n=172)

Table 3 Frequency of antibiotics Resistant Among Clinical and Environmental *Pseudomonas aeruginosa* among the Four Tertiary Hospitals (n=172)

Antibiotics	Resistant Among Clinical Isolates		Resistant Among Environmental isolates	
	Frequency	%	Frequency	%
OFL	76	44.1	15	75
CPX	71	41.2	12	60
AUG	112	68.0	18	90
CN	136	79.0	16	80
AM	137	76.6	18	90
CTX	158	92.1	14	70
CAZ	159	92.5	18	90
S	141	82.0	17	85
IMP	24	13.9	0	0
MEM	23	13.4	1	5
DOR	49	28.5	2	10

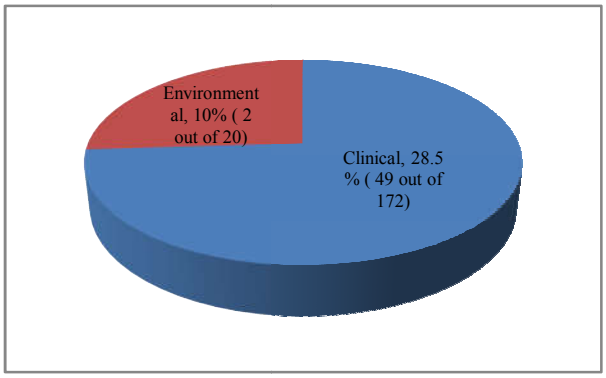

Figure 4 Comparison of Carbapenem resistant among Clinical and Environmental Isolates of *Pseudomonas aeruginosa.*

DISCUSSION

Pseudomonas aeruginosa is one of the most dreadful Gram negative organisms that are commonly found in our health facilities and communities. The high proportion of hospital infections caused by *Pseudomonas aeruginosa* resistant to carbapenem indicates the importance of this organism as a significant cause of infections in our hospitals and communities. Studies done by different authors indicate that intrinsic risk factors such as usage of nasogastric tubes, mechanical ventilation and commencement of antibiotics before laboratory results increased the risk of bacteraemia caused by *Pseudomonas aeruginosa* resistant to carbapenem (6,7,8,9).

In this study, prevalence rates of 12.8% (172 of 1338) and 0.9% (20 of 2230) were recorded for clinical and environmental *Pseudomonas aeruginosa* respectively. The prevalence observed among clinical samples is close to what was reported by Olayinka *et a*l. (10) in Zaria, Nigeria in which prevalence of 10.5% was reported (10). In India, prevalence of *Pseudomonas aeruginosa* infections varies from 10.5% to 32.1% (11,12), while an European study put prevalence of *Pseudomonas aeruginosa* among clinical isolates to be 6.9%(13).

Among the environmental samples, prevalence of 0.9% reported in this study is low, when compared to 52.0 % and 19.5% reported by Minind *et al*. (14) and Nagaba *et al*.(15) respectively. All effort to lay hands on any report on prevalence of *Pseudomonas aeruginosa* among hospital environment isolates in Nigeria was abortive.

Varied prevalence observed from country to country, community to community and even hospital to hospital may be due to the difference in the types of clinical samples collected, types of hospitals and geographical locations. Studies have shown that the prevalence of *Pseudomonas aeruginosa* isolates varies with clinical condition and even the specimen collected (10,11,12,13). No significant association was found between type of samples collected and *Pseudomonas aerginosa*.

Distribution of clinical samples showed wound/pus to predominate with 55.2% (95), while others were 23.3%, 12.8% and 8.7 for urine, ear swabs and blood respectively. Similar studies, where spectrum of samples from different sites yielded growth of *Pseudomonas aeruginosa* were reported globally (11,12,16). High prevalence of *Pseudomonas aeruginosa* was reported in Nigeria among wound samples (17).

The male-female ratio of 50.6: 49.4 reported in this study agrees with the outcome of the work done by Anupurba *et al.,* and Siti Nur Atiquah Idris *et al.,* in which male was found to be having the greater prevalence of *Pseudomonas aeruginosa* of 60% and 57% respectively (18,19). Factors that may be responsible for this skewness include personal habit, nature of work, outdoor activities, exposure to soil and water.

From this research it was observed that *Pseudomonas aeriginosa* infection was higher among hospitalised patients 65.3%. Work done by several authors show that *Pseudomonas aeruginosa* is rarely seen as a normal floral of human but colonization rate may exceed 50% during hospitalization, especially among patients that have impaired immunity probably due to ventilation, tracheostomy, catheters, surgery or severe burns (20, 21, 22).

Pseudomonas aeruginosa showed highest resistant to Cephalosporin in both the clinical and environmental isolates. Several work done in Nigeria and other part of the world supported this findings (4,23, 24,). Although, Ceftazidime has been one of the best and active drugs for the treatment of severe infections caused by *Pseudomonas aeruginosa* in most tertiary healthcare for some time, this study, however, revealed that cephalosporin especially ceftazidime could no longer be used as an agent for emperic treatment in serious infections suspected to be caused by *Pseudomonas aeruginosa*.

Carbapenem antibiotics has the highest susceptibility among the clinical and environmental isolates in this study. However the resistant level found among the clinical isolates is alarming. The result from this study shows that clinical isolates are more resistant to carbapenem than environmental isolates. Even though the findings of this work show that clinical *Pseudomonas aeruginosa* is more resistant to Carbapenem antibiotics than environmental isolate this shows that precautions should be taken to prevent contamination from the environment because the environment could also be a source of carbapenem resistant *Pseudomonas aeruginosa* infections. Necessary measures should be taken; and appropriate policies developed which will dictate the dimension of prescription and dispensary of antibiotics in order to curb the issue of resistance to commonly used antibiotics.

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References

1. Egushi H, Miyamoto T, Kuwahara T and Mitamura Y. Infection conjunctivitis caused by Pseudomonas aeruginosaisolateded from bathroom. BCM Res 2013 6: 245.doi:10/1186/1756-0500-6-245
2. Watanabe, M., Iyobe, S., Inoue, M. and Mitsuhashi, S. Transferable imipenem resistance in Pseudomonas aeruginosa. Antimicrob Agents Chemother. 1991; 35: 147-151.
3. Cornaglia, G., Giamarellou, H. and Rossolini, G. M. Metallo-blactamases: A last frontier for beta-lactams? Lancet Infect Dis. 2011; 11: 381-393.
4. Zubai K.O and Iregbu K.C. Resistance Pattern and Detection of Metallo-beta-lactamase Genes in Clinical Isolates of *Pseudomonas aeruginosa* in a Central Nigeria Tertiary Hospital. *J ClinPract* 2018; 21:176-82
5. Khantayaporn P, Montakantikul P, Mootsikapun P, Thamlikitkul .V and Mullika T. Prevalence and Genotypic Relatedness of carbapenem resistance among multidrug resistance *Pseudomonas aeruginosa* in teriary hospital across Thailand. Annals of Clinical Microbiology and Antimicrobials 2012 11:25
6. Ahmed S.H., Daef E.A and Badary M.S. Nosocomial blood stream infection in intensive care units at Assiut

University Hospitals (Upper Egypt) with special reference to extended spectrum beta-lactamase producing organisms. BMC Res Notes. 2009; 2: 76-86.
7. Aloush V,Navon- Venezia S, Seigman -igra Y, Cabili S, Carmeli Y. Multidrug -resistant *Pseudomonas aeruginosa*. Risk Factor and Clinical Impact. Antimicrob Agents Chemother 2006: 50: 43-8
8. Ohmagari N, Hanna H, Gravsis L, Hackett B, Perego C, Gonzalez V et al. Risk factors for infections with multidrug -resistant *Pseudomonas aeruginosa* in patients with Cancer 2005: 104:205-12
9. Arruda EA, Marinho IS, BoulosM,Sinto SI, Calaffahh, Mendes CM *et al*. Nosocomial infections caused by multidrug resistant *Pseudomonas aeruginosa*. Infect Control Hosp Epidemiol 1999: 20: 620-3
10. Olayinka AT, Olayinka BO, Onile BA. Antibiotic susceptibility and plasmid pattern of *Pseudomonas aeruginosa* from the surgical unit of a university teaching hospital in North central Nigeria. *Int J Med MedSci* 2009; 1:79-83.
11. Srinivas B, Devi DL, Rao BN, Fraser VJ, Kollef MH, *Pseudomonas aeruginosa* and its Antibiogram in a teaching Hospital of Rural set up. *J. Pharm Biomed Sci* 2012; 22; 1-4.
12. Rajat RM, Ninama GL, Mistry K, Parmar R, Patel K, Vegad MM. Antibiotic resistance pattern in *Pseudomonas aeruginosa* species isolated at a tertiary care Hospital, Ahmadabad. *Natl J Med Res* 2012; 2:156-159.
13. Bonza E, San Juan R, Munoz P, Voss A, Kluytmas J. Co-operative Group of the European Study Group on Nosocomial Infections: A European perspective on nosocomial urinary tract infections I. Report on microbiology workload, etiology and antimicrobial susceptibility (ESGNI=003 study). European Study Group on Nosocomial Infection. Clin Microbiology Infect, 2001; 7(10): 523-531
14. Milind Davane, Namdev Suryanshi, Asha Pichare, Basavraj Nagoba. *Pseudomonas aeruginosa* from Hospital Environment, *Journal of Microbiology and Infection*, 2014;4(1):42-43
15. Nagoba BS, Deshmukh SR, Husain RA. Bacteriological analysis of Various environmental sources in a rural hospital, *Ind. J. Med Sci* 1997; 51: 465-469.
16. Mohanasoundaram KM. The antimicrobial resistance pattern in the clinical isolates of *Pseudomonas aeruginosa* in a tertiary care hospital; 2008-2010 (A 3 yr study). *J Clin Diagn Res*. 2011; 5:491-494.
17. Odusanya O O. Antibiotic susceptibility of microorganisms at a General Hospital in Lagos, Nigeria. *J Natl Med Assoc*. 2002; 94: 994 -998.
18. Anupurba S, Battacharjee A, Garg A, Ranjansen M. The antimicrobial susceptibility of *Psuedomonas aeruginosa* isolated from wound infections. Indian J Dermatol. 2006; 51(4):286-288.
19. Siti Nur Atiquah Idris, Mohd Nasir Mohd Desa, MohdNazri Aziz, NiazinTaib. Antimicrobial susceptibility pattern and distribution of EXO U and EXO S in clinical isolates of *Pseudomonas aeruginosa* at a Malaysian hospital. The Southeast Asian Journal of Tropical Medicine 2012; Vol 43:pg 116-123
20. Pollack M. *Pseudomonas aeruginosa*. In: Mandell GL, Dulan R, Bennett JE, editors. Principle and Practices in Infectious Diseases. 4 thed. New York, NY: Churchill Livingstone; 1995. p. 1820-2003
21. Thuong M, Arvaniti K, Ruimy R, de la Salmonière P, Scanvic-Hameg A, Lucet JC, *et al*. Epidemiology of *Pseudomonas aeruginosa* and risk factors for carriage acquisition in an intensive care unit. *J Hosp Infect*. 2003;53:274-282
22. Vallés J, Mariscal D, Cortés P, Coll P, Villagrá A, Díaz E, *et al*. Patterns of colonization by *Pseudomonas aeruginosa* in intubated patients: A 3-year prospective study of 1,607 isolates using pulsed-field gel electrophoresis with implications for prevention of ventilator-associated pneumonia. Intensive Care Med. 2004; 30:1768-1775.
23. Ibukun A, Tochukwu N, Tolu O. Occurrence of ESBL and MBL in clinical isolates of *Pseudomonas aeruginosa* From Lagos, Nigeria. *Journal of American Science*. 2007;3(4):81-85.
24. Igbalajobi O.A, Oluyega A.O, Oladeji A.C, Babalola J.A. Antibiotics Resistance Pattern of *Pseudomonas aeruginosa* Isolates from Clinical Samples In Ekiti State University Teaching Hospital, Ado -Ekiti, Nigeria 2016, 12(4): 1-6

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