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Prevalence of *Klebsiella aerogenes* and multiantibiotic resistant from two teaching hospitals in Portharcourt

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Abstract
Prevalence and development of multi-antibiotic resistant *Klebsiella aerogenes*, are part of the major cause of hospital-acquired infections worldwide. This study investigated the multi-antibiotic resistant (MAR) patterns of *Klebsiella* species, Isolated from FSC from mortuary departments in Rivers state University teaching Hospital and University of Port Harcourt Teaching hospitals for period of 4 months and 7 specimens were collected twice fortnightly. The samples were analyzed by Standard microbiological and Kirby-bauer method, and interpretation of result were according to Clinical Laboratory Standards Institute (CLSI). Reported mean of total coliform counts ranged 5.20 ± 4.89 - 6.62 ± 5.89 x 10^5, total *Klebsiella* counts (TSC) 3.4 ± 2.98 - 3.5 ± 3.0x 10^4cfu/ml from the two mortuaries. The total of 46 isolates consisting of various isolates with relative percentage abundance of *Klebsiella* sp (82.5%), Citrobacter sp (4.3%), unknown sp (6.5%) & Pseudomonas sp (6.5%).The genus, *Klebsiella* is the most predominate with relative abundance of: *Klebsiella aerogenes* (56.5%), *Klebsiella oxytoca* (21.7%), *and Klebsiella pneumoniae* (4.3%). The Klebsiella has a higher relative abundance as a single species than *Klebsiella pneumoniae* & *oxytoca* isolated from both morgue departments in the two hospitals. The susceptibility pattern of the isolates to antibiotic are Gentamycin (60%)> Ciprofloxacin (25%)> Ofloxacin (10%) and Streptomycin (5%) Respectively. Antibiotic resistant gene evaluation was observed that *Klebsiella aerogenes* possess plasmid that carried the following gene: $^{bla}_{CTX\text{-}M}$ type enzymes of beta-latamase, $^{bla}_{AAC}$, $^{bla}_{OXA}$ & $^{bla}_{TEM}$ in this study. *Klebsiella aerogenes* had presented the highest multiantibiotic resistance with a MAR index of more than 1.00. From the above challenge; Approach like educating the public on the danger associating with indiscriminate dispose of corpses and unprotected touching of fresh swabbed corpses and the adverse effect posed to the public.

Keywords: total coliform count, antibiotic-resistant strains, *Klebsiella aerogene* antibiotic resistant gene, Nigeria

Introduction
Prevalence and development of multi-antibiotic resistant *Klebsiella aerogenes*, and other Gram-negative bacteria which are the major cause of hospital-acquired infections worldwide, had been increasing. Klebsiella sp. had become more resistant to different classes of antibiotics, and the treatment of infections caused by this bacteria had developed into a challenge in both developed and developing countries**.** *Klebsiella aerogenes* belonging to the family Enterobacteriaceae, genus Klebsiella, previously Known as *Enterobacter aerogenes*, gram negative bacilli, facultative anaerobe, Non-Motile, and a Polysaccharide capsule. The member of the *Klebsiella* genus typically have types of antigens on their cell surface: Lipopolysaccharide (O-Antigen) and the other is capsular polysaccharide (K-Antigen) which are both contributed to pathogenicity. They survives within plant (wood & sawdust), soil, surface water, gastrointestinal tract and sewage and poorly colonized on human skin, but colonization rate increase directly proportional to the length of time spends. They can survive for extended period of time. They are mostly spread through person to person contact and less spread by contamination in the environment, they are also found in hospital setting with other healthcare associated infections cause human nosocomial infections. Large amount of *Klebsiella* infection however, are associated with hospitalization as opportunistic pathogens, primarily attack immunocompromised individuals who are hospitalized and suffer from severe underlying disease.In particular, the medically most important *Klebsiella* species, its accounts for a significant proportion of hospital-acquired urinary tract infections, pneumonia, septicemias, and soft tissue infections. The principal pathogenic reservoirs for transmission of Klebsiella are the gastrointestinal tract and the hands of hospital personnel. Because of their ability to spread rapidly in the hospital environment. Klebsiella poses high threat to clinicians as a cause of community-acquired bacterial pneumonia, occurring particularly in chronic alcoholics and showing characteristic radiographic abnormalities due to a severe pyogenic infection which has a high fatality rate if untreated.

Multi-antibiotic-resistant *Klebsiella aerogenes* [1] especially those in Morgue and neonatal wards, are often form new types of strains, extended-spectrum-β-lactamase (ESBL) producers. Are usually plasmid mediated, Sometimes as a result long-term hospitalization before dead the incidence of ESBL-producing strains among hospital acquired *Klebsiella* isolates has been steadily increasing during this study. Multi-antibiotic- resistant strains [1] has been reported with related increase in infectivity across all ages admitted in the hospital wards. Some studies has revealed the epidemiology of

antibiotic-resistant profiling for of *Klebsiella pneumonia* over 10years period in referral hospital, Kenya. They are highly responsible for urinary tract infection and high incidence in some specific group at risk (neuropathic bladders and diabetes mellitus condition), underlying diseases and certain environmental factors

The limitations on the therapeutic options demand new strategies for the management and control of new strains *Klebsiella* hospital infections. While the different typing methods are useful epidemiological tools for infection control, recent findings about Klebsiella virulence factors have provided new insights into the pathogenic strategies of these bacteria. Klebsiella pathogenicity factors such as capsules or lipopolysaccharides are presently considered to be the main molecule for vaccine production, efforts that may serve as immunological infection control measures also as virulence factors to produce diseases.

Materials and Methods

Collection of Specimen (fresh swabbed corpses)

The study area for the research work is Mortuary department of University of Port Harcourt teaching hospital (UPTH) and Rivers state University Teaching hospital. mortuary departments in Rivers state University teaching Hospital and University of Port Harcourt Teaching hospitals for period of 4 months; A total 112 specimens were collected ;7 fresh swabbed corpses were collected at each time twice a month from university of Port Harcourt, this process were repeatedly conducted in Rivers state University Teaching Hospital. The external skin surface was swabbed in duplicate with sterile swab stick aseptically from Mortuary Department of the two teaching hospitals in a container containing a transport media and cold chain system (cabinet), then was transported immediately to laboratories.

Microbiological /Bacteriological Analysis.

The analysis was done in (i) University of Port Harcourt, Microbiology laboratory (ii) Reality scan diagnostics services laboratory (iii) Rivers state University, Microbiology laboratory for Bacteriological analysis: As soon as the sample arrived in the laboratory, some were preserved in a suitable condition and others were analyzed immediately through several microbiological steps, namely: a ten (10) fold serial dilution of the samples, the serial dilution process involves the use of sterile physiological saline also known as diluent solution of 8.5 % sodium chloride and distilled water, to reduce the viable microbial population in sample to be plated. An aliquot (0.1ml = 100ul) was pipette with automated pipette from the dilution of choice of the investigative swabbed sample 9ml saline diluent (thus, making it 10ml) also referring as dilution at 10^{-4} dilution factor. The dilution factor (10^{-5}) and (10^{-4}) of Swabbed saline preparation were used for the analysis.

Media Used

Eosin Methylene Blue Agar (EMB), CLED (cysteine Lactose Electrolyte Deficient) Simmons Citrate agar,

Modified media Used

M-FC Agar (Tryptose,proteose peptone, Yeast extract,Lactose, Bile salt mixture, sodium chloride, aniline blue,Agar,1litre),Mac-Conkey (peptone 20gm,lactose monohydrate 10gm,bile Salt1.5gm.Nacl 5gm,Neutral red 0.3g,crystal violet 0,001mg, Agar 13.5 mg & 1litre). All selective media used were prepared according manufacturer instruction, example in the case of Mac-conkey medium were 13.4g was suspended in 280L of distilled in 500_{cc} of conical flask then stoppered and sterilized by autoclaving at 121 ^{0}C for 15min and after was allow to cool at 50 ^{0}C. Some of the medium were supplemented with 450ml of 10% *myo-inositol* and 300µl of 100mg/ml carbenicillin.

Inoculation of Samples

Direct spread and streaking method) onto different prepared media of prepared and incubation followed. Other steps involves; observation of yielded colonies, enumeration of total coliform count, isolation for pure culture isolates and isolates preservation. The characterization and identification of isolated colonies which is aim to determine the genus & species of the isolate via biochemical and sequencing.

Enumeration of Total Coliform Load

All yielded colonies were observed on the growth medium after 24hrs–48hrs, and counted together in colony forming unit per milliliter and calculated with dilution factor. Pure culture isolates were obtained from mixed isolates through an isolation technique, flaming of wire loop on bursen burner, were allowed to cool, touch the colony to be isolated with the loop, follows by inoculating on a different dry, Non-contaminated growth medium.

Antibiotic Susceptibility Test

24 hours old pure culture recovered isolates suspected to be *Klebsiella* sp, according to biochemical characterization result were subjected to different antibiotics. Bacterial suspension were prepared by gradually emulsified bacterial cells in a sterile peptone broth to match 0.5mcfarland turbidity standard composed of Barium chloride (0.05ml) and Sulphuric acid (9.95ml), before was poured onto a prepared Mueller Hinton Agar (was prepared according to manufacturer instruction of 20 mililitre were poured aseptically in a sterile newly glass petri dish and allowed to solidify). And allow to settle before introduction of conventional antibiotic sensi disk on the seeded bacterial medium. All the *Klebsiella* isolates suspension were subjected to the following antibiotics: Ceftriaxone(CTR)-30 µg, Cefixime (CFX))- 5 µg, Gentamicin (GN)-10 µg, ciprofloxacin (CPX)-5 µg, ofloxacin(OFX)-5 µg, ceftazidime (CAZ)-30 µg, cefpodixime (KNX)-10 µg, Cefixime (CXM)-5 µg, Nitrofurantoin (NIT)-300 µg, streptomycin (S)-30 µg, cefuroxime (CRX)-30 µg, Augmentin (AUG)-30 µg obtained from Abtek Biological Ltd LOT SC07/P & LOT TF05/P respectively, manufactured in United Kingdom. Antibiotic susceptibility test had been carried out by using disk diffusion method, and the interpretation of results of the zones of inhibition had accorded with Clinical Laboratory Standards Institute (CLSI).

Determination of MAR Index

Determination of MAR index in which the number of antibiotics an isolate is resistant to (a) is divided by the total number of the antibiotics used in the study (b). The calculating

formula is shown below:

Statistical Analysis

Data collected was subjected to analyses and presented in a tabular chart or graphic format according to Okolie (2007). The data involves several statistical methods such as;

1. Measure of central tendency
2. Measure of variability
3. Percentage determination
4. Descriptive analysis.

Amplification and Sequence Analysis of Bacterial 16s RNA DNA Extraction

The isolate was allow to grown overnight in Meat extract Peptone broth and an aliquot was harvested to use in DNA Extraction was achieve using Zr Fungal/Bacterial DNA Miniprep (Manufactured by Zymo research cat number: D6005) 2mLs of bacterial cells broth were added to a ZR BashingTM Lysis Tube and 750ul Lysis Solution were also added to the tube secured with fitted bead of 2 ml tube holder assembly and process at maximum speed for > 5 minutes. Centrifuge the ZR Bashing BeadTM Lysis Tube in a microcentirifuge at > 10,000 x g for 1 minute, then pipette 400ul of the supernatant and Transfer up to to a Zymo-SpinTM IV Spin Filter (orange top) in a Collection Tube and centrifuge at 7,000 x g for 1 minute,1,200 ul of Fungal/Bacterial DNA Binding Buffer to the filtrate in Collection Tube from Step 4.Transfer 800 ul of the mixture from Step 5 to a Zymo-SpinTM IIC Column in a Collection Tube and centrifuge at 10,000 x g for 1 minute,7. Discard the flow through from the Collection Tube and repeat Step 6.Add 200 ul DNA Pre-Wash Buffer to the Zymo-Spin TM IIC Column in new Collection Tube and centrifuge at 10,000 x g for 1 minute, 9. Add 500 ul Fungal/Bacterial DNA Wash Buffer to the Zymo-SpinTM IIC Column and centrifuge at 10,000 x g for 1 minute, transfer the Zymo-SpinTM IIC Column to a clean 1.5 ml micro centrifuge tube and add 100ul (35 ul minimum) DNA Elution Buffer directly to the column matrix. Centrifuge at 10,000 x g for 30 seconds to elute the DNA. The PCR mix is made up of 12.5µL of Taq 2X Master Mix from New England Biolabs (M0270); 1µL each of 10µM 16SrRNA gene forward primer (16SF GTGCCAGCAGCCGCGCTAA) and reverse primer (16SRNA: AGACCCGGGAACGTATTCAC); 3µL of DNA template and then made up with 7.5µL Nuclease free water.

Amplification Cycling

Initial denaturation at 94˚C for 5mins, followed by 36 cycles of denaturation at 94˚C for 30sec, annealing at 56˚C for 30secs and elongation at 72˚C for 45sec. Followed by a final elongation step at 72˚C for 7 minutes and hold temperature at 10 ˚C forever.

Results

Based on colonies formation and morphological characterization on this study is was practically observed that Klebsiella utilize inositol nitrate of aniline as sole carbon and nitrogen sources, basically most of the media with inositol and aniline strictly encourages the growth Klebsiella species, others like Macconkey allow both *Klebsiella* and non-*Klebsiella* bacteria.the Non Klebsiella grows formed smaller colonies to distinguish from Klebsiella Bacteria.

Table 1: The Degree of Coliform Growth on the Different Selective Media.

S/N	Coliforms Type	No. Isolate	Growth rate on Selective media				
			EMB	SCA	MCICA	M-FC	CLED
1	*Klebsiella aerogenes*	26	++	+++	+	+++	++
2	*Klebsiella oxytoca*	10	++	++	+++	++	+++
3	*Klebsiella pneumoniae*	2	+++	+	+	+	++
6	Unknown	3	+	++	+	+	-
7	*Enterococcus faecalis*	2	-	-	+	-	++
8	*Pseudomonas aeroginosa*	3	+++	+	++	-	++

Key**:** Mild growth (+), Moderate growth (++), Heavy growth (+++), Eosin Methylene blue (EMB), Simmon-Citrate gar (SCA), Macconkey-inisitol-carbenicillin (MCICA), Modified Faecal coliforms Agar (M-FC).

Table 2: Microbial population of fresh swabbed corpses

S/N	Microbial isolates	Location	
		UPTH	RSUTH
1	Total Coliform Count (TCC) ($x10^5$) cfu/ml	6.62 ± 5.89	5.20 ± 4.89
2	Total Klebsiella count (TKC) ($x10^4$) cfu/ml	3.4 ± 2.98	3.5 ± 3.0

UPTH

University Port Harcourt Teaching Hospital, RSUTH; Rivers State University Teaching Hospital, cfu/ml; Colony forming unit per millilitre, ±; Values are mean of triplicates determinations, each variable was Used independent of the statistically significant or insignificant variables presented as P> 0.05=probability of null hypothesis,> =Greater than p= probability.

Microbial population enumeration shows total coliforms count and Total *Klebsiella* recovered from two Teaching hospitals located in Port Harcourt metropolis, in this study, total coliforms load and Total Klebsiella enumeration on Mac-conkey Agar (MCIC) obtained from UPTH were significantly higher to RSUTH number after an unbiased experimental enumeration.

Table 3: Biochemical Characterization

S/N	Obtained Isolates	Biochemical Test Parameters															
		NO. of Iso NO	VP	MR	In	Ur	Citr	LDC	Oxd	EsH	Mot	Catalase	Gram rx	Glucose	Lactose	Mannito	
1	*Klebsiella oxytoca*	10	+	-	+	+	+	+	-	+	-	+	-	+	+	+	

2	*Pseudomonas aeruginosa*	3	-	-	-	-	+	-	+	-	-	+	-	-	-	+
3	*Sarratia marcescens*	3	+	-	-	+	+	+	-	+	+	+	-	+	-	+
4	*Klebsiella Pneumoniae*	2	+	-	-	+	+	+	-	+	-	+	-	+	+	+
5	*Enterococcus faecalis*	2	+	-	-	-	-	-	-	+	-	-	+	+	+	+
6	*Klebsiella aerogenes*	26	+	-	-	-	+	+	-	+	+	+	-	+	+	+

Keys:Ur=Urease,VP=Voges Proskauer, M=methy red, Citr=citrate,Oxd= oxidase, rx= Reaction, In=indoles LDC=lysine deoxycarboxylase, Mot=Motility, Iso =Isolates, EsH=Esculin hydrolysis,+ =Positive,- =Negative.

In this study, all procedures used in carried out the biochemical evaluation of each isolates was strictly in line with manufacturer instruction at different experimental condition. It was observed that all isolates of enterobacteriaceae were positive to sugar fermentation test conducted on three parameters, glucose, lactose and Mannitol sugar. The biochemical result of *Klebsiella aerogenes* were similar to *Pseudomonas aeruginosa* except the negative of oxidase and positive of Voges Proskauer (VP) and so on in other isolates.

Table 4: Frequency of occurrence of *Klebsiella* sp isolates in Mortuary Fresh Swabbed Corpses.

No of isolates	UPTH		RSUTH		
	Morgue swabbed=	Percentage of abundance (%)	Morgue swabbed	Percentage of bundance (%)	Total No. (%)
Klebsiella aerogenes	16	64	10	50	26(56.5)
Klebsiella oxytoca	4	16	6	30	10(21.7)
Klebsiella pneumoniae	0	0	2	10	2(4.3)
Unknown	3	12	0	0	3(6.5)
Citrobacter sp	1	4	1	5	2(4.3)
Pseudomonas aeroginosa	2	8	1	5	3(6.5)
Total	26	100	20	100	46(99.8)
Total No. (%)	26(54.3)		20(43.5)		

Antibiogram Profiling

Table 5: Antibiogram pattern of *Klebsiella aerogenes*

University of portharcourt teaching hospital(Upth)				
Mortuary FSC				
Antibiotics	**Conc. (µg)**	**Resistant n (%)**	**Intermediate n (%)**	**Susceptible n (%)**
Ofloxacin(OFL)	5ug	3(18.8)	4(25)	9(56.3)
Augumentin (AUG)	30(µg)	15(93.8)	1(6.3)	0(0.00)
Gentamycin(GEN)	30(µg)	2(12.5)	2(12.5)	12(75)
Cefuroxime (CRX)	5(µg)	13(81.3)	3(18.8)	0(0.00)
Streptomycin (S)	30(µg)	12(75)	4(25)	0(0.00)
Cefixime (CFX)	5 (µg)	10(62.5)	3(18.8)	1(6.3)
Ceftazidime(CAZ)	30(µg)	16(100)	0(0.00)	0(0.00)
Ciprofloxacin (CPR)	10 (µg)	2(12.5)	3(18.8)	11(68.8)
Cefpodoxime (KNX)	10(µg)	2(12.5)	1(6.3)	13(81.3)
Nitrofurantoin (NIT)	300(µg)	7(43.8)	7(43.8)	2(12.5)

Key: n (%)=Number and percentage of occurrence,S= Sensitivity, I= intermediate, R= Resistant,(µg)= Microlitre, conc. = concentration, FSC=Fresh swabbed corpses.

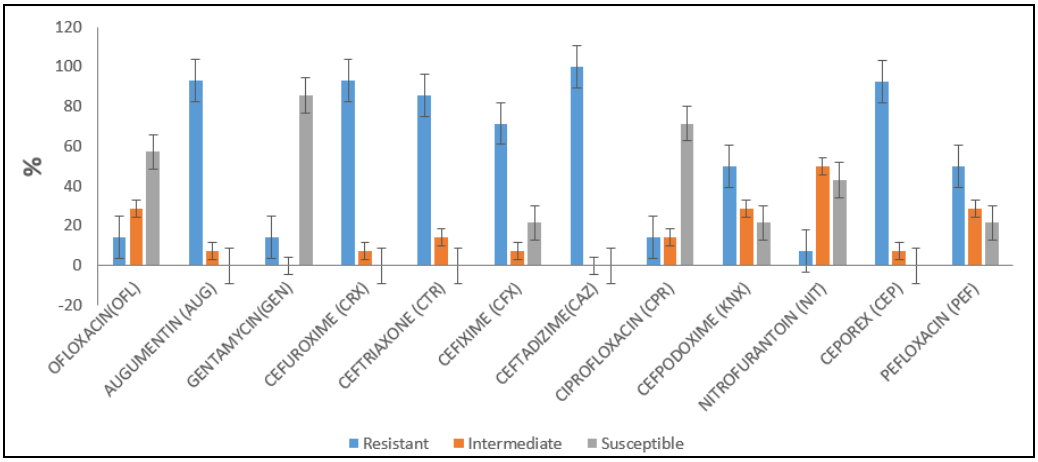

Fig 1

Antibiogram Profiling

Table 6: Antibiogram pattern of *Klebsiella aerogenes*

Rivers state university teaching hospital(Rsuth)				
Mortuary FSC				
Antibiotics	**Conc. (µg)**	**Resistant n (%)**	**Intermediate n (%)**	**Susceptible n (%)**
Ofloxacin(OFL)	30ug	0(0.0)	2(20)	8(80)
Augumentin (AUG)	30(µg)	10(100)	0(0.00)	0(0.00)
Gentamycin(GEN)	30(µg)	0(0.00)	2(30)	7(70)
Cefuroxime (CRX)	5(µg)	8(80)	1(10)	1(10)
Streptomycin (S)	30(µg)	3(30)	1(10)	6(60)
Cefixime (CFX)	5 (µg)	7(70)	1(10)	2(20)
Ceftadizime(CAZ)	30(µg)	10(100)	0(0.00)	0(0.00)
Ciprofloxacin (CPR)	10 (µg)	1(10)	2(20)	7(70)
Cefpodoxime (KNX)	10(µg)	2(20)	3(30)	5(50)
Nitrofurantoin (NIT)	300(µg)	3(30)	3(30)	4(40)

Key: n (%) =Number and percentage of occurrence, S= Sensitivity, I= intermediate, R= Resistant, (µg) = Microlitre, conc. = concentration, FSC=Fresh swabbed corpses.

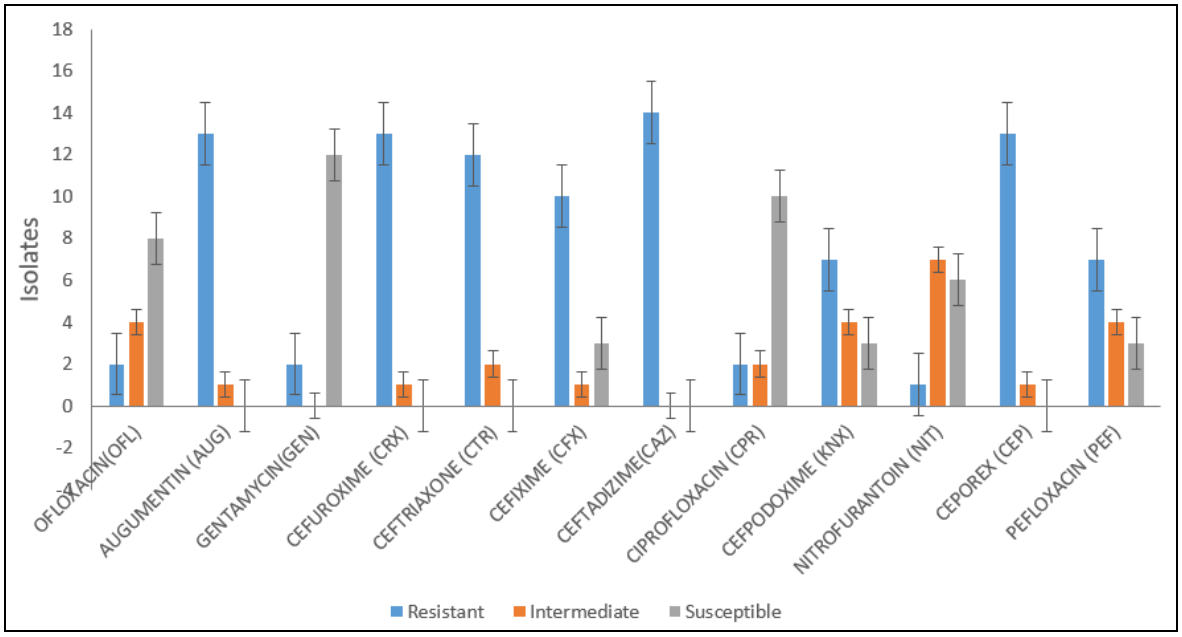

Fig 2

Table 7: Multiple antibiotics resistance index of *Klebsiella aerogenes* isolate from UPTH & Rsuth FSC.

Mar index	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**
Klebsiella aerogenes	0(0.00)	0(0.00)	0(0.00)	2(14.3)	9(64.3)	3(21.4)	0(0.00)	0(0.00)	0(0.00)

Key: MAR**:** Multiple Antibiotic Resistance, (n %): Number of percentage of Bacteria occurrence.

Molecular Characterization of the Isolates

The presumptive Isolates, *Unknown* sp, *Klebsiella* sp, *Pseudomonas* sp, *Citrobacter freundii* obtained after biochemical analysis were subjected to Molecular analysis through PCR technology. On molecular evaluation, 16r RNA of all the isolates were subjected to investigation that revealed the same or similarity match and evolutionary distance during mega BLAST search for highly similar sequence from NCBI data base. Phylogenetic characterization of all the presumed 16SrRNA isolates were analyze and identify, they are *Klebsiella aerogenes*. The suspected 16SrRNA of *Citrobacter* sp was identified as *Klebsiella aerogenes* with 96% 16SrRNA similarity. With accession number of MK014300.

The suspected 16SrRNA of *Pseudomonas aeruginosa* was also identified as *Klebsiella aerogenes* with 82% 16SrRNA compatibility with accession number of MK026852**.**

The suspected 16SrRNA of *Citrobacter fraudii* was identified as *Klebsiella aerogenes* with 81% 16SrRNA relatedness with accession number of HM582426.

Although, all presumed isolates, *Citrobacter* sp,*Unknwn* sp, *pseudomonas* sp, *Klebsiella* sp, *Citrobacter* sp which were obtained and characterized phenotypically and biochemically. Among the obtained isolates *Klebsiella* sp was accepted during 16SrRNA Molecular characterization.

All the molecular identified strains were resistant to most third generation cephalosporin, Corpses of the two morgue, UPTH

and RSUTH colonized with most cephalosporin resistant Klebsiella sp. 46 Isolates obtained from both UPTH and RSTH harbored 100% of Ceftaziximе resistant *Klebsiella aerogenes*,80-81% Cefuroxime resistant *Klebsiella aerogenes*,70% of cefixime resistant *Klebsiella aerogenes* and 100% of Augumentin resistant *Klebsiella aerogenes*.in 48 samples obtained from RSUTH harbored 100% of Ceftadixime resistant *Klebsiella aerogenes*, 90% Cefuroxime resistant *Klebsiella aerogenes*,70% of cefixime resistant *Klebsiella aerogenes* and 60% of Augumentin resistant *Klebsiella aerogenes*. Metagenomic study revealed that the recovered *Klebsiella aerogenes* from the two sampling unit were encoded with the following gene, SHA, AAC,CTX-M, TEM & OXA that carries beta-lactamase enzymes,a broad spectrum against beta lactam ring antibiotics. TEM-10 gene are most special gene responsible for *Klebsiella aerogenes* to be resistant to Ceftazidime, cefixime,augumentin and cefuroxime resistance.they are mostly plasmid mediated resistance gene that extended spectrum beta-lactamase (ESBL).

Discussion

A total of 46 Isolates were isolated on aggregate of the selective media used from the fresh swabbed, corpses were observed that *Klebsiella aerogenes* grew well on MCIC [6] almost all the media except on SCA[25], inhibit the growth as a result of modification with antibiotics that, aim to determine the rate at which MCIA [6] can support the growth of non-Klebsiella sp.The supplemented CLED inhibited most of the non-Klebsiella sp and support the growth of *Klebsiella aerogenes*.M-FC agar inhibited the growth of non-*Klebsiella* sp due to the present of Rolisic acid, colonies similarity on the different selective agar was a challenge to distinguish the phenotypic characterization but biochemical characterization were used. Example, *Klebsiella* sp colonies appeared large on all the selective media (MCIC, EMB, SCA, M-FC & CLED) and Non *Klebsiella* sp formed smaller colony, the colonies are obviously different in color formation on different media as a result of content base formulation of the media.

Some good selective medium were considered to detect, control and monitor a specific group of bacteria (Enterobacteriaceae), MCIC is one of the selective medium used in selection of *Klebsiella* sp [12], the disadvantage of this medium is the present of carbenicillin antibiotic [25] has the capacity to inhibit the growth of some Strains of *Klebsiella* [14] sometimes the unwanted strains still grow due to inactivation of inhibitory antibiotic used. But unfortunately *Escherichia coli* grow profusely than other enterobacteriaceae, as a result plasmid mediated Amp^r gene. Among bacteria obtained *Klebsiella aerogenes* were about 56.5% seconded by *Klebsiella oxytoca* of 21.7% and Unknown sp of 6.5%. SCA and M-FC is an alternative medium that can encourage and yield a distinguish colonies when plated, this medium contain a major carbon source, C itrate, which *Klebsiella* utilized optimally for their growth. The colony produced is according to its capacity to utilize it carbon source and the color changes formed is the ability of the organism to withstand the P^H indicator in cooperated. CLED contain deoxycholate compound used to inhibit gram positive bacteria, was observed that CLED agar produced weak growth of *Klebsiella aerogenes* when compared with SCA & MCIC. This means CLED are sensitive to most strains of *Klebsiella* that reduced their growth propensity. Inversely, CLED is not advisable for the cultivation *Klebsiella aerogenes.*

The load of *Klebsiella aerogenes* bacteria isolate obtained in sample on fresh swabbed corpses from UPTH (university of Port Harcourt Teaching Hospital), were significantly similar and slightly higher to RSUTH (River State University Teaching Hospital),this revealed the increased of Enterobacteriaceae growth from fresh swabbed corpses before prepare for embalming.

The slight significant difference and the significant increase observed in the Total Coliform count obtained between the both hospitals mortuary departments satisfy permissible counts of Coliforms bacteria on fresh swabbed corpses In this study total coliform bacteria count isolated in the samples obtained from the two hospitals were significantly moderate and related which satisfy the total coliform counts of fresh swabbed corpses.

Bacterial Occurrence

The result of the bacterial population of fresh swabbed corpses from both teaching hospitals as presented in Table 2 showed that the total coliform count for University of Port Harcourt Teaching Hospital and Rivers state University Teaching hospitals ranged from 5.20-6.62 $x10^5$cfu/ml. the variation in the coliform population of fresh swabbed corpses across the two Teaching hospitals can be a result of associated hospital acquired infection and other factors, such as follows; poor sanitary protocol, the increase practicing of unprofessional handling (during ambulation), contamination by the mortuary workers, or receiving of these bodies terms to enhance the chances of Enterobacteriaceae population infested indicated.there was no significant difference ($P \leq 0.05$) in total coliform count.

Molecular process/ evaluation on all the presumed isolates, sequencing unveiled *Klebsiella aerogenes* to have highest percentage of occurrence probably is as a result contamination of faecal material or the infection responsible for the disease and the presence of wide range of natural environments (plants, soil & GIT) the high prevalence level of *Klebsiella aerogenes* may be due shut-down, internal temperature changed, poor health/physical care. This may be due to hospital acquired (nosocomial pathogens) and other environmental factor as contact source, *Klebsiella oxytoca* was identified as the second most abundance isolate of enterobacteriaceae in sample from both sampled units, fresh swabbed corpses in a moderate number of varied percentage, the identified isolates are usually an inhabitant of soil, may have being originated from poor hospital setting, colonized in tap water and mostly found in intensive care unit, rodents dropping etc. the *Klebsiella aerogenes, Klebsiella oxytoca* & *Citrobacter* sp varied in percentage in a relative abundance obtained from both FSC according to the molecular sequencing evaluation. The prevalence appearance of *Klebsiella aerogenes* are normal inhabitants of intestinal tract, genital tract and less common in oral cavity also the major cause of urinary tract infection. The most prevalent of all isolates molecularly identified.

The Mueller hinton agar were used based on noninterference

to antibiotic molecules during Antibiogram –Resistiblity patterns test [22] study of *Klebsiella aerogenes* isolated from UPTH Mortuary shown that resistance in order of Augmentin (30 µg)> ceftadizime (30 µg) >cefuroxime (30 µg) >Nitrofurantoin (300 µg). Susceptibility were in order as Ofloxacin (10 µg)> Ciprofloxacin (10 µg) >Gentamycin (10 µg).the number of resistance were higher than the sensitivity, *Klebsiella aerogenes* isolated from RSUTH Morgue shows 100% Susceptibility to ofloxacin (5 µg) this study.

It was observed that *Klebsiella aerogenes* exhibit strong activity against third generation cephalosporin. In-vitro Antibiogram testing revealed resistant to third generation cephalosporin (ceftadizime, cefuroxime and cefixime) which are strong beta-lactam ring antibiotics as result of presence of the following genes; SHA, AAC, TEM & OXA obtained after the molecular evaluation gross abused of antibiotics in the study was noted.

Conclusion

The result obtained from this research experiment conclude that;

The study result underscored that *Klebsiella aerogene* is evenly associated with fresh dead bodies

Swab Samples collected from a fresh child corpse's harbored high Enterobacteriaceae member than an adult corpses with a credible number as a result of increasing number of third generation cephalosporin *Klebsiella aerogenes* resistant in the children ward of university teaching hospitals in Port Harcourt

Total coliform count were higher in University of Port Harcourt teaching hospital than Rivers state university teaching Hospital, this indicate the level of practice and management system.the microbial of fresh corpses skin had high heterotrophic bacteria and low coliforms count above healthy skin.

The study indicates a high bacterial load on the skin of fresh corpse's before deposition that revealed microbial colonization during hospitalization or contamination during ambulation in University of Harcourt Teaching and Rivers state University Teaching Hospital, and the prevalence state of microorganism among the two teaching hospitals is of public health concern when compared to the recommended and approved standard for laboratory effluent to be discharged.

Isolates, *Klebsiella aerogenes* obtained from fresh corpses are 100% susceptible to Gentamicin, Ciprofloxacin and Cefpodoxime followed by Ofloxacin with intermediate susceptibility. Isolates, *Klebsiella aerogenes* obtained from fresh corpses have 100% resistance features to Augumentin, Ceporex. Rifampicin, Cefuroxime, Cefixime, Pefloxacin, Ceftazixime and Septrin. In the study area. The study obtained plasmid mediated cephalosporin resistant Klebsiella aerogenes (PMCRK) through a several batches of Antibiogram profiling. Some isolated *Pseudomonas aeruginosa* Strains poses high biological risk due to the concentrated resistance gene,CTX-M TEM,SHV & OXA. The presence of genes confer pseudomonas to be more resistance to various antibiotics including broadspectrum. Some sub-strains of *Klebsiella aerogenes* lack CTX-M gene, one of the beta-lactamases encoding gene, which allows the strain to be more susceptible to beta-lactam ring antibiotics.The study also revealed that dead bodies from hospitals, deposited in the mortuary are associated with nosocomial bacterial group with multi-antibiotics resistant strains.

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