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The Phylogency (Evolutionary Relationship) and Antibiogram of *Acinetobacter baunmanni* Isolated from Tertiary Health Institutions in Rivers State Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The increasing rate of *Acinetobacter baumannii* in recent time as a major pathogen associated with hospital acquired infections is burdensome. This has resulted to significant morbidity and mortality predominantly among the immunocompromised patients, prolonged hospitalization with increased cost. The global burden of *Acinetobacter baumannii* infections is still unclear as a result of inadequate comprehensive data particularly in developing countries such as the case in Africa. There are inadequate extensive works on the phylogency of *Acinetobacter baumannii* and the region of this study is not well represented. The cross-sectional hospital based study investigated the phylogency (evolutionary relationship) and antibiogram of *Acinetobacter baunmanni* isolated from Tertiary Health Institutions in Rivers State, Nigeria. Study included individuals within the study coverage and those outside the selected facilities were excluded. Ethical approval and informed written consent was obtained. Both primary and secondary data were used. Isolation and identification involved culturing, biochemical and molecular assay; were conducted sequentially. Statistical Package for Social Science version 21 was used to perform descriptive and inferential statistics at 0.05 level of significance. The study has proved the concept about the phylogency (evolutionary relationship) between *Acinetobacter baumanii* with *Klebsiella pneumoniae* and *Enterobacter pneumonia*. Antibiogram based on the minimum zone of inhibition CPX had the

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highest mean±SD 24.222±1.7845 whereas, CXM recorded least 6.478±7.7092. Cephalosporin and Tetracycline class of drugs appear to be more resistant while Fluoroquinolone, and Amioglycoside are sensitive. The researcher recommends strict antibiotic surveillance and prevention as well as control measures. Also, supplementary study involving molecular assay with large sample size is recommended and extensive study of the virulence genes.

Keywords: Phylogency; evolutionary relationship; antibiogram; Acinetobacter baunmanni; tertiary health institutions; Rivers State; Nigeria.

1. INTRODUCTION

The genus *Acinetobacter* has demonstrated considerable modification in taxonomy over decades. There are other species but *Acinetobacter baumannii* appears to be the most major representative and a significant hospital pathogen globally. Over the years, the clinical significance of *Acinetobacter baumannii* thrived by its notable capacity to up-regulate or acquires resistance determinants, contributing significantly to the global threat as pertains antibiotic resistance. The emergence of *Acinetobacter baumannii* in recent time as a major pathogen associated with Nosocomial infections is burdensome and this has resulted to significant morbidity and mortality predominantly among the immunocompromised patients, prolonged hospitalization with increased cost (Bashir et al., 2019; Muhammad et al., 2018; Mirnejad et al., 2018).

The global burden of *Acinetobacter baumannii* infections is still unclear as a result of inadequate comprehensive data particularly in developing countries such as the case in Africa (Egwuenu et al., 2018) although some have measured the burden with estimates of 35% - 45% with mortality rate of 26% (Muhammad et al., 2018; Xie et al., 2018). In addition, *Acinetobacter baumannii* strains resistant to all known antibiotics have now been reported, signifying a sentinel event that should be acted on promptly by the international health care community. Acting in synergy with this emerging resistance profile is the eccentric ability of *Acinetobacter baumannii* to survive for prolonged periods throughout a hospital environment, thus potentiating its ability for nosocomial spread. The organism commonly targets the most vulnerable hospitalized patients, those who are critically ill with breaches in skin integrity and airway protection. As reported from reviews dating back to the 1970s (Glew et al., 1977), hospital-acquired pneumonia is still one of the most common infection caused by this organism. However, in more recent times, infections involving the central nervous system, skin and soft tissue, and bone have emerged as highly problematic for health institutions. Based on the severity of the problem posed by this organism, there is need for this study owing to the fact that, there is scarcity of data showing evolutionary relationship of *Acinetobacter baunmanni*. Phylogenetic study is timely needed to successfully combat the antibiotic resistance menace of this organism in this era.

The study was a cross-sectional hospital based study which investigated the phylogency (evolutionary relationship) and antibiogram of *Acinetobacter baunmanni* isolated from Tertiary Health Institutions in Rivers State, Nigeria.

2. METHODS

2.1 Study Area

The area of the study is Port Harcourt, the capital and major city of Rivers State. It is located on Latitude 4^0 46' 38"N longitude; $7^0$00' 48" with an elevation above sea level: 16m. It has an estimated population of 1,867,000 and covers an area of 367km^2 and lies along Bonny river. There is high level of oil and gas activities within the region. Main seasons are dry and rainy seasons. Main occupation is fishing and farming.

The study was conducted with samples collected from Rivers State University Teaching Hospital (RSUTH) and University of Port Harcourt Teaching Hospital (UPTH), two tertiary healthcare facilities serving catering for patients across Rivers State and some adjoining states.

2.2 Study Population

The study population of the study consisted of 380 subjects ranging from 3 – 65 years both male and female. Sample size was calculated according to Daniel (1999) using a prevalence rate of 14% for Acinetobacter in the University College Hospital, Ibadan (UNICEF, 2009).

2.3 Sample Collection and Laboratory Analysis

Each sample was examined macroscopically. Also, microscopic examination, culture, and biochemical identification were performed. Gram staining, biochemical test such as catalas, oxidas and motility test were performed according to Lone et al *(*2009) and Gupta et al*. (*2015) following isolation.

2.4 Molecular Identification

The isolates phenotypically identified as *Acinetobacter* were subjected to molecular identification using DNA extraction (Boiling method), DNA quantification and 16S rRNA. Also, amplification and Phylogenetic Analysis were performed.

2.5 Phylogenetic Analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using MAFFT. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou & Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method (Jukes & Cantor, 1969).

2.6 Statistical Analysis

Statistical Package for Social Science version 21, was used to analysis data after collation into excel spread sheet. Descriptive and inferential statistics at 0.05 level of significance were analysed. Frequency, percentage, mean, standard deviation, and t test were the specific test statistics performed.

3. RESULTS

The study investigated phylogency (evolutionary relationship) and antibiogram of *Acinetobacter baunmanni* isolated from Tertiary Health Institutions (RSUTH and UPTH) in Rivers State, Nigeria. The study specifically isolated, characterized, and identified bacterial species from urine, wound, and aspirate samples from the two main tertiary hospitals (RSUTH and UPTH) in Rivers State, Nigeria.

3.1 Evolutionary Distances between the Bacterial Isolates

The obtained 16s rRNA sequence from the isolate produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The 16S rRNA of the isolate showed a percentage similarity to other species at 100%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolates within the *Acinetobacter, Klebsiella* and *Enterobacter* sp revealed a closely relatedness to *Acinetobacter baumanii, Klebsiella pneumoniae* and *Enterobacter pneumoniae* (Fig. 1).

3.2 Antibiogram Pattern of Acinobacter

3.2.1 Descriptive statistics and mean comparison of antibiogram pattern-minimum zone of inhibition (mm)

Comparatively, mean differences were quantitatively computed for three categories namely location, gender, and sample type using independent sample t test.

The antibiogram pattern on Table 1 shows descriptive statistics of Minimum Zone of Inhibition (mm) by Location. A total of nine (9) samples obtained from RSUTH = 3, and UPTH = 6 were tested against fifteen (15) antibiotics (drugs). Based on the minimum zone of inhibition CPX had the highest mean±SD 24.222±1.7845mm whereas, CXM recorded least 6.478±7.7092mm.

Furthermore, the comparative analysis of the difference in the antibiogram patterns of isolates from RSUTH and UPTH showed that, out of the fifteen (15) drugs used in this study between the two locations sampled for all the drug panel, only Phenicol showed an evidence of statistical significant difference between RSUTH and UPTH ($t=2.895$, $df = 7$, $N =9$, $p = 0.02$) while others were not significant. This means that the antibiogram patterns of isolates from RSUTH and UPTH only varied when tested only for phenicol (CH), others showed no variation hence, the same. See details on Table 1.

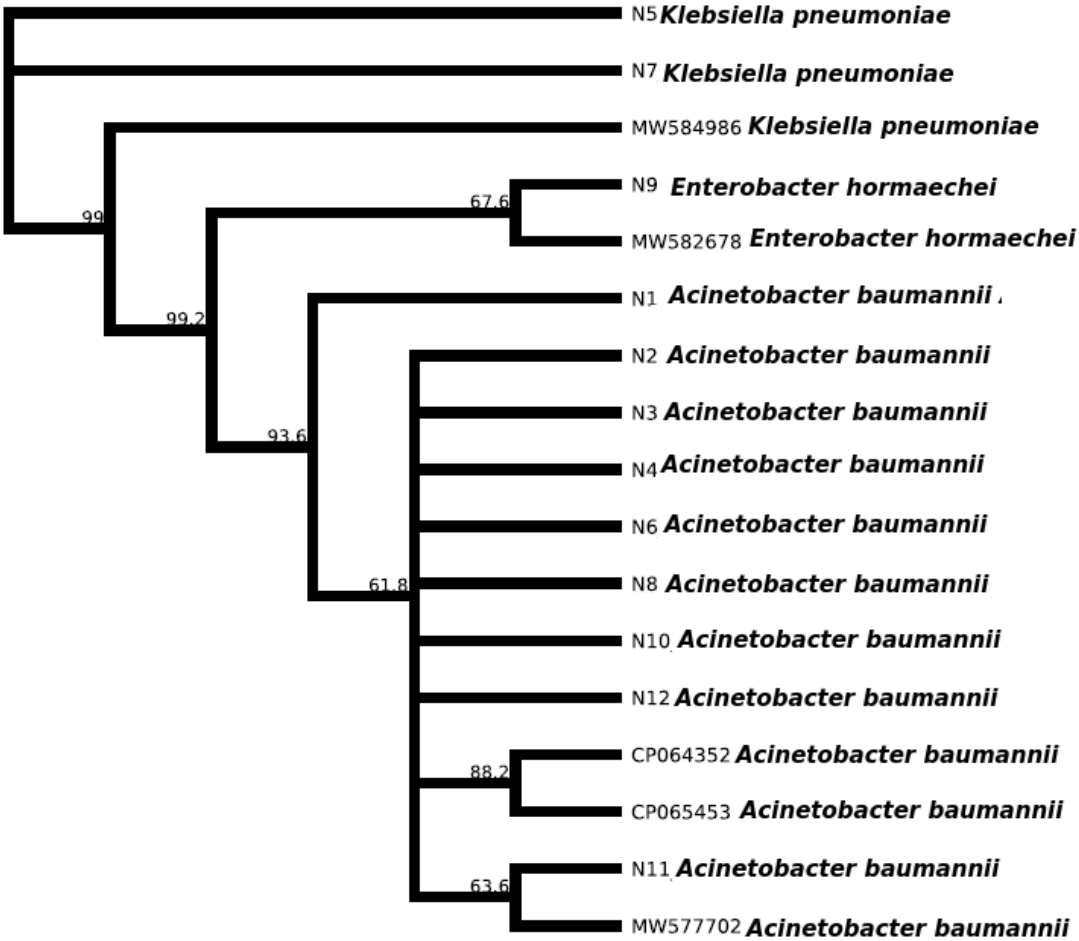

Fig. 1. Evolutionary distances between the bacterial isolates

Table 1. Mean comparison of antibiogram (minimum zone of inhibition) by location

Drugs	RSUTH N=3 (mm)	UPTH N=6 (mm)	Total N=9 (mm)	t-test	df	p-value
AM	12.200±10.9563	12.400±8.0489	12.333±8.3970	-.032	7	.976
CTX	24.200±11.4904	14.917±9.9785	18.011±10.8066	1.258	7	.249
CXM	9.933±10.7221	4.750±6.1935	6.478±7.7092	.944	7	.376
CP	10.233±10.8445	9.033±7.7389	9.433±8.1971	.194	7	.852
CH	17.467±2.1733	14.233±1.2660	15.311±2.1900	**2.895**	**7**	**.023**
CPX	24.100±1.4731	24.283±2.0527	**24.222**±1.7845	-.136	7	.896
CTZ	12.867±3.7072	13.283±2.8046	13.144±2.8975	-.191	7	.854
D	3.667±6.3509	8.600±4.6117	6.956±5.4277	-1.350	7	.219
ER	17.833±3.8991	11.683±9.2741	13.733±8.1861	1.072	7	.319
@GT	16.733±.3512	17.400±1.8974	17.178±1.5466	-.584	7	.578
LV	20.433±.7371	18.950±1.9947	19.444±1.7812	1.212	7	.265
NX	17.500±.4583	17.917±.6853	17.778±.6241	-.937	7	.380
OFX	24.033±.6658	23.883±1.2007	23.933±1.0087	.197	7	.849
PFX	23.333±.9292	22.383±1.0439	22.700±1.0595	1.327	7	.226
ST	16.633±2.6388	16.483±3.4661	16.533±3.0422	.065	7	.950

P<0.05=Sig=Significant, p>0.05= NA=Not Significant. AM: Amoxicillin/Clavulanic acid CTX: Ceftriaxone CXM: Cefuroxime CP: Cephalexin CH: Chloramphenicol CPX: Ciprofloxacin CTZ: Cotrimoxazole D:Doxycyclinecycline ER: Erythromycin GT: Gentamycin LV: Levofloxacin NX: Norfloxacin OFX: Ofloxacin PFX: Pefloxacin ST: Streptomycin

Similarly, Table 2 presents descriptive statistics of Antibiogram (Minimum Zone of Inhibition) by Gender. For the females OFX had the highest 24.250±.8849mm and D was the lowest 4.883±5.5312. While the males had CPX 24.267 ±2.2898mm as the highest while CXM = 6.967±7.8679mm appeared least. Summarily, CPX = 24.222±1.7845mm was the highest irrespective of the gender whereas, CXM = 6.478± 6.478mm was the least.

Table 2 likewise points out the mean comparison showing t test of Antibiogram Pattern (Minimum Zone of Inhibition) by Gender. Study finding revealed no evidence of statistical significant discrepancy between male and female groups in almost all drugs tested except Tetracycline (D) [t=-1.848, df=7, p=0.04) following the analysis. By implication, there is no considerable variance in the antibiotic profile between the two groups when tested against fifteen drug panels with the exception of Tetracycline as initially postulated hence, all others were equivalent. See Table 2 for more.

In same vein, Table 3 showed the mean, standard deviation and t test of Antibiogram (Minimum Zone of Inhibition) by Sample. In this case, CPX = 24.471±1.7356mm and CXM were the highest and least drugs respectively. The wound swab sample showed OFX = 23.850±.7778mm and CXM = 10.450±7.1418mm for highest and lowest in terms of minimum zone of inhibition. On the whole, the total report revealed CPX = 24.222±1.7845mm and CXM=6.478±7.7092 for most efficacious and least respectively.

In addition, mean comparison based on sample type for Antibiogram Pattern (Minimum Zone of Inhibition). The result proved absence of statistical significant differences (p>0.05) for all organisms originating from urine and wound when tested against the fifteen (15) drugs. See Table 3 for detailed presentation of result.

3.2.2 Frequency and percent distribution of antibiogram pattern (resistant and sensitivity)

Table 4, showed overall Frequency Distribution of Antibiogram Pattern (Resistant and Sensitivity) as observed in this study. The investigation showed trimodal distribution 8(88.9%) for the resistant for CXM, CP and D (in essence, Cephalosporin and Tetracycline class of drugs) while five (5) drugs had zero resistants namely; CPX, GT, NX, OFX, and PFX (Fluoroquinolone, and Amioglycoside class of drug). Based on sensitivity report, four (4) drugs had the highest frequency 9(100%) with regard to sensitive namely; @GT, NX, OFX, PFX (Amioglycoside and Fluoroquinolone class of drug).

Table 2. Mean comparison showing t test of antibiogram pattern (minimum zone of inhibition) by gender

Drugs (mm)	Mean ±SD Female N=6	Mean ±SD Male N=3	Mean ±SD Total N=9	t-test	df	p-value
AM	12.350±7.9334	12.300±11.1665	12.333±8.3970	.008	7	.994
CTX	19.417±12.0245	15.200±9.3744	18.011±10.8066	.526	7	.615
CXM	6.233±8.3735	6.967±7.8679	6.478±7.7092	-.126	7	.903
CP	7.967±9.6018	12.367±4.3501	9.433±8.1971	-.737	7	.485
CH	15.583±2.5039	14.767±1.6862	15.311±2.1900	.502	7	.631
CPX	24.200±1.7309	24.267±2.2898	24.222±1.7845	-.049	7	.962
CTZ	13.267±2.7245	12.900±3.8588	13.144±2.8975	.168	7	.872
D	4.883±5.5312	11.100±1.6462	6.956±5.4277	**-1.848**	**7**	**.042**
ER	11.350±9.2165	18.500±2.1378	13.733±8.1861	-1.284	7	.240
@GT	17.800±1.4339	15.933±.9713	17.178±1.5466	2.002	7	.085
LV	19.333±2.1201	19.667±1.1590	19.444±1.7812	-.249	7	.811
NX	17.717±.7387	17.900±.4000	17.778±.6241	-.393	7	.706
OFX	24.250±.8849	23.300±1.1000	23.933±1.0087	1.412	7	.201
PFX	23.050±1.1167	22.000±.5196	22.700±1.0595	1.509	7	.175
ST	15.833±3.5240	17.933±1.2503	16.533±3.0422	-.973	7	.363

P<0.05=Sig=Significant, p>0.05= NA=Not Significant. AM: Amoxicillin/Clavulanic acid CTX: Ceftriaxone CXM: Cefuroxime CP: Cephalexin CH: Chloramphenicol CPX: Ciprofloxacin CTZ: Cotrimoxazole D:Doxycyclinecycline ER: Erythromycin GT: Gentamycin LV: Levofloxacin NX: Norfloxacin OFX: Ofloxacin PFX: Pefloxacin ST: Streptomycin

Table 3. Descriptive statistics of antibiogram (minimum zone of inhibition) by sample

Drugs (mm)	Mean±SD Urine N=7	Mean±SD Wound N=2	Mean±SD Total N=9	t-test	df	p-value
AM	10.586±8.6162	18.450±4.7376	12.333±8.3970	-1.200	7	.269
CTX	17.329±12.2887	20.400±3.6770	18.011±10.8066	-.334	7	.748
CXM	5.343±7.9987	10.450±7.1418	6.478±7.7092	-.808	7	.446
CP	9.043±9.2161	10.800±4.8083	9.433±8.1971	-.251	7	.809
CH	15.300±2.4055	15.350±1.9092	15.311±2.1900	-.027	7	.979
CPX	24.471±1.7356	23.350±2.3335	24.222±1.7845	.763	7	.470
CTZ	12.643±2.9849	14.900±2.4042	13.144±2.8975	-.968	7	.365
D	6.043±5.9081	10.150±.0707	6.956±5.4277	-.936	7	.380
ER	12.471±8.9213	18.150±2.8991	13.733±8.1861	-.850	7	.423
@GT	17.414±1.6597	16.350±.9192	17.178±1.5466	.843	7	.427
LV	19.271±1.9423	20.050±1.3435	19.444±1.7812	-.520	7	.619
NX	17.800±.7095	17.700±.2828	17.778±.6241	.187	7	.857
OFX	23.957±1.1193	23.850±.7778	23.933±1.0087	.124	7	.905
PFX	22.814±1.1950	22.300±.0001	22.700±1.0595	.580	7	.580
ST	16.214±3.3712	17.650±1.6263	16.533±3.0422	-.563	7	.591

Table 4. Overall Frequency Distribution of Antibiogram Pattern (Resistant and Sensitivity)

Class of Drug	Drugs	Frequency (%)	
		Resistant	Sensitive
Penicillin	AM	7(77.8%)	2(22.2%)
Cephalosporin	CTX	5(55.6)	4(44.4%)
Cephalosporin	CXM	8(88.9%)	1(11.1%)
Cephalosporin	CP	8(88.9%)	1(11.1%)
Phenicol	CH	6(66.7%)	3(33.3%)
Fluoroquinolone	CPX	0(0.0%)	2(22.2%)
Folate Pathway Inhibitor	CTZ	6(66.7%)	3(33.3%)
Tetracycline	D	8(88.9%)	1(11.1%)
Macrolides	ER	7(77.8%)	2(22.2%)
Amioglycoside	@GT	0(0.0%)	9(100.0%)
Fluoroquinolone	LV	1(11.1%)	8(88.9%)
Fluoroquinolone	NX	0(0.0%)	9(100.0%)
Fluoroquinolone	OFX	0(0.0%)	9(100.0%)
Fluoroquinolone	PFX	0(0.0%)	9(100.0%)
Amioglycosides	ST	2(22.2%)	7(77.8%)

Table 5 pictures the Frequency Distribution of Antibiogram Pattern (Resistant and Sensitivity) by Location. The study recorded different distributions for the various locations. RSUTH had bimodal occurrence for resistant of D and ER 3(100%) each and six (6) drugs had null resistance 0 (0.0%) namely; CPX, @GT, LV, NX, OFX, and PFX. Report from UPTH showed, bimodal distribution 6(100%) for resistant for CXM and CP drugs while four drugs were most sensitive 6(100%) namely; CPX, NX, OFX, and PFX.

Table 6 shows Frequency Distribution of Antibiogram Pattern (Resistant and Sensitivity) by Gender. Study findings reported bimodal resistant 6(100%) for isolates from female subject against D and ER. Nonetheless, CPX, @GT, LV, NX, OFX, and PFX had no resistant 0(0.0%) rather showed 6(100%) sensitive as seen. The isolates from the male subject had similar resistant of bimodal occurrence but against CXM and CP 3(100%) but dissimilarity exist in nine (9) drugs namely; CPX, D, ER, GT, LV, NX, OFX, PFX, and ST; no resistant 0(0%) was reported for these drugs. Besides, the most resistant became the null sensitive and the zero resistant appeared to be the most sensitive as observed.

Table 5. Frequency distribution of antibiogram pattern (resistant and sensitivity) by location

Drugs	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)
	RSUTH N=3		UPTH N=6	
	Resistant	Sensitive	Resistant	Sensitive
AM	2(66.7%)	1(100.0%)	5(83.3%)	1(16.7%)
CTX	2(66.7%)	(100.0%)	3(50.0%)	3(50.0%)
CXM	2(66.7%)	(100.0%)	6(100.0%)	0(0.0%)
CP	2(66.7%)	(100.0%)	6(100.0%)	0(0.0%)
CH	1(33.3%)	2(66.7%)	5(83.3%)	1(16.7%)
CPX	0(0.0%)	3(100.0%)	0(0.0%)	6(100.0%)
CTZ	2(66.7%)	(100.0%)	4(66.7%)	2(33.3%)
D	3(100.0%)	0(0.0%)	5(83.3%)	1(16.7%)
ER	3(100.0%)	0(0.0%)	4(66.7%)	2(33.3%)
@GT	0(0.0%)	3(100.0%)	0(0.0%)	3(100.0%)
LV	0(0.0%)	3(100.0%)	1(16.7%)	5(83.3%)
NX	0(0.0%)	3(100.0%)	0(0.0%)	6(100.0%)
OFX	0(0.0%)	3(100.0%)	0(0.0%)	6(100.0%)
PFX	0(0.0%)	3(100.0%)	0(0.0%)	6(100.0%)
ST	1(33.3%)	2(66.7%)	1(16.7%)	5(83.3%)

Table 6. Frequency distribution of antibiogram pattern (resistant and sensitivity) by gender

Drugs	Frequency(%)	Frequency (%)	Frequency(%)	Frequency(%)
	Female N=6		Male N=3	
	Resistant	Sensitive	Resistant	Sensitive
AM	5(83.3%)	1(16.7%)	2(66.7%)	1(33.3%)
CTX	3(50.0%)	3(50.0%)	2(66.7%)	1(33.3%)
CXM	5(83.3%)	1(16.7%)	3(100.0%)	0(0.0%)
CP	5(83.3)	1(16.7%)	3(100.0%)	0(0.0%)
CH	4(66.7%)	2(33.3%)	2(66.7%)	1(33.3%)
CPX	0(0.0%)	6(100.0%)	0(0.0%)	3(100.0%)
CTZ	4(66.7%)	2(33.3%)	2(66.7%)	1(33.3%)
D	6(100.0%)	2(66.7%)	0(0.0%)	1(33.3%)
ER	6(100.0%)	1(16.7%)	0(0.0%)	2(66.7%)
@GT	0(0.0%)	6(100.0%)	0(0.0%)	3(100.0%)
LV	1(16.7%)	5(83.3%)	0(0.0%)	3(100.0%)
NX	0(0.0%)	6(100.0%)	0(0.0%)	3(100.0%)
OFX	0(0.0%)	6(100.0%)	0(0.0%)	3(100.0%)
PFX	0(0.0%)	6(100.0%)	0(0.0%)	3(100.0%)
ST	2(33.3%)	4(66.7%)	0(0.0%)	3(100.0%)

Table 7. Frequency distribution of antibiogram pattern (resistant and sensitivity) by sample

Drugs	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)
	Urine N=7		Wound	
	Resistant	Sensitive	Resistant Sensitive	Sensitive
AM	6(85.7%)	1(14.3%)	1(50.0%)	1(50.0%)
CTX	4(57.1%)	3(42.9%)	1(50.0%)	1(50.0%)
CXM	6(85.7%)	1(14.3%)	2(100.0%)	0(0.0%)
CP	6(85.7%)	1(14.3%)	2(100.0%)	0(0.0%)
CH	5(71.4%)	2(28.6%)	1(50.0%)	1(50.0%)
CPX	0(0.0%)	7(100.0%)	0(0.0%)	2(100.0%)
CTZ	5(71.4%)	2(%28.6)	1(50.0%)	1(50.0%)
D	6(85.7%)	1(14.3%)	2(100.0%)	0(0.0%)

Drugs	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)
	Urine N=7		Wound	
	Resistant	Sensitive	Resistant Sensitive	Sensitive
ER	6(85.7%)	1(14.3%)	1(50.0%)	1(50.0%)
@GT	0(0.0%)	7(100%)	0(0.0%)	2(100.0%)
LV	1(14.3%)	6(85.7%)	0(0.0%)	2(100.0%)
NX	0(0.0%)	7(100.0%)	0(0.0%)	2(100.0%)
OFX	0(0.0%)	7(100.0%)	0(0.0%)	2(100.0%)
PFX	0(0.0%)	7(100.0%)	0(0.0%)	2(100.0%)
ST	2(28.6%)	5(71.4%)	0(0.0%)	2(100.0%)

Table 7 is about Frequency Distribution of Antibiogram Pattern (Resistant and Sensitivity) by Sample specifically urine and wound swab. Study outcome revealed that six (6) drugs were the most resistant 6(85.7%) namely; AM, CXM, CP, D, and ER while five (5) drugs were the most sensitive 7(100%) namely; CPX, GT, NX, OFX, and PFX.

4. DISCUSSION

4.1 Phylogeny/Evolutionary Relationship

The phylogenetic finding from this study is in consonance with earlier theories and pragmatic studies about *Acinetobacter baumannii* with respect to classification and evolutionary relationship. *Acinetobacter baumannii* belongs to the genus Acinetobacter. From the recent taxonomic classification, *Acinetobacter baumannii* has maintained γ-proteobacteria, from the family Moraxellaceae and order Pseudomonadales (Nemec et al., 2016). Further classification placed *Acinetobacter baumannii* to Acinetobacter calcoaceticus-baumannii complex group and this group comprises of four different Acinetobacteria namely: A. baumannii, Acinetobacter pittii, Acinetobacter nosocomialis, and Acinetobacter calcoaceticus (Pourabbas et al., 2016; Nemec et al*.,* 2016; Muhammad et al*.,* 2018).

Acinetobacter, Klebsiella and *Enterobacter* sp revealed a closely relatedness to *Acinetobacter baumanii, Klebsiella pneumoniae* and *Enterobacter pneumonia* as observed in other study (Nemec et al., 2016). The classified organism - *Acinetobacter baumannii* is particularly associated with infections in the hospital and its environment therefore, termed a high risk nosocomial pathogen with huge disease burden.

Furthermore, the isolation of *Acinetobacter baumanii* in this study confirms the presence of this pathogen in the hospital as established in earlier studies (Muhammad et al., 2018; Pourabbas et al., 2016; Nemec et al*.,* 2016). *Acinetobacter baumanii* being common in the hospital and its environment; different areas and units within the hospital have become a niche and studies have proven that this organism is harboured there leading to its spread (Muhammad et al., 2018). This hospital acquired infection *Acinetobacter baumanii* is a concern for clinicians and public health at large. Although, the presence of this organism in a host may be harmless for individuals with strong-uncompromised immunity; it is not the same for immunosuppressed patients. This is very important as many people in the hospital have low immunity and are susceptible to this opportunistic infection resulting from *Acinetobacter baumanii*.

Based on evolutionary relationship obtained in this study, *Acinetobacter baumanii,* and *Klebsiella pneumonia* are closely related as well as *Enterobacter pneumonia.* This relatedness has shown in the characteristic features of these organisms including their capabilities to cause disease and spread. Anthonella et al. (2014) in a review showed similar trends in the isolation density plus increasing multidrug resistant strains of *Acinetobacter baumanii, and Klebsiella pneumonia* in hospitals*.* Also, *Acinetobacter baumanii,* and *Klebsiella pneumonia* possess the potential to cause respiratory (lung) infection such as pneumonia. Besides, both organisms are well known to cause nosocomial infection [1]. The emergence of *Acinetobacter baumannii* and *Klebsiella pneumoniae* strains in the hospital environment has been associated with the presence of multiple genetic elements, virulence factors and the ability to form biofilms (Paola et al., 2016). Furthermore, prior studies have implicated this closely related organisms to cause increase profile of hospital pathogens both intra and inter hospital transmission of resistant strains (Anthonella et al., 2014) hence, a clonality

of alert microorganisms in addition to antibiotics consumption monitoring for effective control of infections. Besides, these related organisms share similar traits such as heterogeneous property which also aids biofilm formations. The biofilm characteristic of the pathogens is crucial. Species of *Acinetobacter* are good in the formation of biofilm therefore known as one of the biofilm producing bacteria. This biofilm production aids the organisms in surviving adverse environmental conditions like that seen in the hospital environment [2]. Also, virulence factors and other genetic properties in these related organisms share similarities (Paola et al., 2016).

4.2 Antibiogram Pattern of *Acinetobacter baumanii*

The present study shared dissimilarity with Nwadike et al*.* [3] study in terms of antibiogram pattern. Ciprofloxacin, Ofloxacin, gentamicin were among the sensitive drugs examined in this study; this however, contradicts Nwadike, Ojide, & Kalu [3] work which listed these drugs as those the isolates showed resistance. Furthermore, the antibiogram pattern observed here is not equal to the analysis of Ayenew et al*.* [2]. Ayenew et al*.* [2] also support that the species are becoming increasingly resistant to nearly all routinely administered antimicrobial agents, including aminoglycosides, fluoroquinolones, and broad-spectrum β-lactams. Nonetheless, this disagreed with this present finding. Specifically, isolates showed high resistance to penicillin similar to the observation of Pal et al*.* (2017). Furthermore, Cephalosporin class of antibiotic which was resistance in this study showed no discrepancy with the work of Odewale et al*.* (2016) and Pal et al*.* (2017). Distinction exists between this study and a recent study [21] which reported high to moderate resistance for ceftazidime, ciprofloxacin, and gentamycin. However, while the organism was highly susceptible to these drugs can be linked to some factors.

Also, the antibiogram pattern in this study shared agreement and opposition with another older study (Alkali et al*.,* 2019). Isolates were sensitive to ciprofloxacin as seen in this study but resistance to ceftazidime and ceftriaxone is in opposition to this study. Victor et al*.* [4] observed that the isolates were poorly susceptible to ciprofloxacin, this is not in consonance with this study. A. *baumannii* was observed to be resistant to commonly administered antibiotics and this finding is similar to previous studies according to literatures [4]. Besides, the study findings on resistance to the commonly prescribed cephalosporins is not consistent with results from different geographical area and population, which reported resistance to cefotaxime, ceftazidime, and cefepime [5,6]. Likewise, the study of Victor et al*.* [4].

Although, Shete et al [7] stated that *Acinetobacter* spp have the tendency to readily develop resistance to third generation cephalosporins and fluoroquinolones; this was not observed in this study. These classes of drugs were the least resistant as observed based on empirical evidence. Also, this study is in contrary to the study of Boon et al*,* [8] which demonstrated complete resistance for ceftriaxone but not the case in this study. This confers protective advantage to people within the region of this study.

The high resistance rates found in this study can also be attributed to abuse of antibiotics. This tradition may have mounted selective pressure resulting to the emergence of multidrug resistant strains. Also, the presence and inheritance of resistance plus virulence genes contributes [9,10]. The broad spctrum intrinsic and acquired resistance determinants that have emerged in recent time for *Acinetobacter baumanii* have reasonably drawn attention to this direction.

This study disagreed with a Northern Iran based study which revealed high resistance to fluoroquinolone class of antibiotics Ghasemian [11]. In like manner, Reguero and colleagues [6] in Colombian hospitals provided a report which contradicts the findings here. In addition to the finding of Victor et al*.* [4], Islahi et al*.* [12] and Akalin et al*.* [13] reports did not support the findings in this research; ciprofloxacin resistance was high compared to the present observation. The contrary variations noticed in this present research and other previous researches outlined are an indication of a drift. Two different studies were conducted at different period in the same facility, Karimi and colleagues [14] reported no resistance to ciprofloxacin but Victor et al. [4] reported very high resistance confirming a drift in antibiogram pattern over the years.

The susceptibility of *A. baumannii* resistance to ciprofloxacin agrees with Karimi et al*.* [14]. The fluoroquinolone class of antibiotic which was highly potent in this research could mean that there was no likely mutation. In essence, the DNA remained structurally unmodified because a

change in gyrase subunits by *gyrA* and *parC* gene mutations is capable of eliciting resistance. Besides, if the protein of the outer membrane is unaltered there might be no development of resistance genes as well as drug accumulation in the intracellular environment resulting to susceptibility [15].

The result of the antibiogram pattern is indicative of antibiotic resistance menace constituted by this organism. The rate of antibiotic resistance make *Acinetobacter baumanii* infections pose therapeutic challenge thereby resulting to elevated number of infection and death. *Acinetobacter baumanii* has posed a threat increasing disease burden; the goal should be on complete eradication or bringing it to the barest using targeted measures [16-21].

5. CONCLUSION

The study has proved the concept about the phylogency (evolutionary relationship) between *Acinetobacter baumanii* with *Klebsiella pneumoniae* and *Enterobacter pneumonia*. Also, confirmed the similarity in the antibiogram patterns of different *Acinetobacter baunmanni* irrespective of the sources.
Antimicrobial susceptibility is not dependent on sources such as sample type, gender and locations. Cephalosporin and Tetracycline class of drugs appear to be more resistant while Fluoroquinolone, and Amioglycoside were sensitive. *Acinetobacter baumanii* from the area of this study are high risk and multidrug resistant organisms as observed. The presence of some resistance genes could be attributed to the high resistance hence, the need for resistance profiling.

6. RECOMMENDATION

The researchers recommend strict antibiotic surveillance and prevention as well as control measures. Targeted measures, such as the isolation of patients and temporary closure or even reconstruction of wards are required to combat *Acinetobacter baumanii* infection and transmission.

Also, further investigation can be made to evaluate of *Acinetobacter baumanii* to carbapenem resistance. Besides, supplementary study involving molecular assay with large sample size is recommended including a revisit of the study area to ascertain any change in antibiogram pattern and distribution rate. In addition, virulence gene should be studied in-depth as much have not been done in this aspect.

7. LIMITATION OF THE STUDY

Although Colistin (polymyxin E) is an antibiotic used as a last-resort for multidrug-resistant gram negative infections' including *Acinetobacter*, this agent was not tested in the present study. Further molecular characterization of *Acinetobacter* exploring the resistant genes examined just three and one virulence gene. All strains were not tested against carbapenem.

Also, molecular analysis was limited in sample size; only twelve samples were subjected to molecular assay because of cost. There are missing data and the socio-demographics of study participants including history, life style, and antibiotic use were not captured.

ETHICAL APPROVAL AND CONSENT

Ethical approval was obtained from various unit heads and health institutions used. Also, informed consent was sought and eligible participants recruited into the study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chhatwal P, Ebadi E, Schwab F, Ziesing S, Vonberg RP, Simon N, Gerbel S, Schlüter D, Bange FC, Baier C. Epidemiology and infection control of carbapenem resistant Acinetobacter baumannii and Klebsiella pneumoniae at a German university hospital: a retrospective study of 5 years (2015–2019). BMC infectious diseases. 2021 Dec;21(1):1-0.

2. Ayenew Z, Tigabu E, Syoum E, Ebrahim S, Assefa D, Tsige E. Multidrug resistance pattern of Acinetobacter species isolated from clinical specimens referred to the Ethiopian Public Health Institute: 2014 to 2018 trend anaylsis. PloS one. 2021 Apr 29;16(4):e0250896. Available:https://doi.org/10.1371/journal.pone.0250896
3. Nwadike VU, Ojide CK, Kalu EI. Multidrug resistant acinetobacter infection and their antimicrobial susceptibility pattern in a nigerian tertiary hospital ICU. Afr J Infect Dis. 2014;8(1):14-8.
4. Victor Moses Musyoki, Moses Muia Masika[1], Winnie Mutai[1], Gitau Wilfred, Antony Kuria, Felista Muthini. Antimicrobial susceptibility pattern of Acinetobacter isolates from patients in Kenyatta National Hospital, Nairobi, Kenya. Pan Africn Medical Journal. 2019;33:146,
5. Omer MI, Gumaa SA, Hassan AA, Idris KH, Ali OA, Mustafa M et al. Prevalence and resistance profile of Acinetobacter baumannii clinical isolates from a private hospital in Khartoum, Sudan. Am J Microbiol Res. 2015;3:76-9.
6. Reguero MT, Medina OE, Hernández MA, Flórez DV, Valenzuela EM, Mantilla JR. Antibiotic resistance patterns of Acinetobacter calcoaceticus-A baumannii complex species from Colombian hospitals. Enferm Infecc Microbiol Clin. 2013;31(3):142-6..
7. Shete V, Ghadage D, Muley V, Bhore A. Multidrug resistant Acinetobacter ventilator associated pneumonia. Lung India. 2010;4:217–221.
8. Boon HK, Yasmin AH, Mohd MY, Kwai LT. Antimicrobial susceptibility profiling and genomic diversity of multidrug-resistant acinetobacter baumanniiisolates from a Teaching Hospital in Malaysia. Jpn J Infect Dis. 2011;64:337–340.
9. Federico P, Hujer AM, Hujer KM, Brooke KD, Philip NR, Bonomo RA. Global Challenge of Multidrug-Resistant Acinetobacter baumannii. Antimicrob agents chemother. 2007;51:3471–3484.
10. Souli M, Galaini I, Giamarellou H. Emergence of extensively drug-resistant and pandrug resistant Gram negative bacilli in Europe. Eurosurveillance. 2008;13:1–10
11. Ghasemian R, Ahanjan M, Fatehi E, Shokri M. Prevalence and antibiotic resistance pattern of Acinetobacter isolated from patients admitted in ICUs in Mazandaran, Northern Iran. Glob J Heal Sci. 2016;8:112-9.
12. Islahi S, Ahmad F, Khare V, Mishra N, Yaqoob S, Shukla P et al. Prevalence and resistance pattern of Acinetobacter species in hospitalized patients in a tertiary care centre. J Evol Med Dent Sci. 2014;3(17):4629-35.
13. Akalin H, Sinirta M, Ocakolu G, Yilmaz E, Heper Y, Kelebek N et al. Nosocomial Acinetobacter pneumonia: treatment and prognostic factors in 356 cases. Respirology. 201621(2):363-9.
14. Karimi PN, Bururia JM, Odhiambo PA, Bamugune BK, Museve GO. Enterobacteriaceae members are primary inhabitants of the lower gastrointestinal tract of man and animals. East Cent Africa J Pharm Sci. 2009;12:42-5.
15. Rabirad N, Mohammadpoor M, Lari AR, Shojaie A, Bayat R. Antimicrobial susceptibility patterns of the gram-negative bacteria isolated from septicemia in Children's Medical Center, Tehran, Iran. J Prev Med Hyg Italy. 2014;55(1):23-6.
16. Antonella A., Martina B., Quattrocchi A., Andrea M., Aldisio E, Marchese EEA. Antibiotic trends of Klebsiella pneumoniae and Acinetobacter baumannii resistance indicators in an intensive care unit of Southern Italy, 2008–2013. Antimicrob Resist Infect Control. 2015;4:43.. DOI: 10.1186/s13756-015-0087-y
17. Awad E, Osman I, El N, El M. High prevalence of multidrug-resistant Acinetobacter species in Khartoum Intensive Care Units (ICUs). Am J Res Commun. 2015;3(2):35-42.
18. Biglari S, Hanafiah A, Ramli R, Rahman MM, Nizam Khaithir TM. Clinico-epidemiological nature and antibiotic susceptibility profile of Acinetobacter species. Pakistan J Med Sci. 2013;29(2):469-73.
19. Seifert H. An increasing threat in hospitals: multidrug-resistant Acinetobacter baumannii. Nat Rev Microbiol. 2007;5:939–954.
20. Sohail M, Rashid A, Aslam B, Waseem M, Shahid M, Akram M et al. Antimicrobial susceptibility of Acinetobacter clinical isolates and emerging antibiogram trends for nosocomial infection management. Rev Soc Bras Med Trop. 2016;49(3):300-4.

21. Xie R, Zhang XD, Zhao Q, Peng B, Zheng J. Analysis of global prevalence of antibiotic resistance in Acinetobacter baumannii infections disclosed a faster increase in OECD countries. Emerging microbes & infections. 2018; 7(1):31.
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