Distribution Pattern of Hospital Isolated *Acinetobacter baunmanni* from Selected Tertiary Health Institutions in Rivers State Nigeria

ABSTRACT

The ubiquitous nature of *Acinetobacter baumannii* has made the Gram-negative, non-motile bacterium to be associated with hospital-acquired infection at the global level and with respect to antimicrobial resistance, it has been categorized among the most dangerous multiple drug-resistant (MDR) pathogens globally and enlisted in the "priority 1: critical" pathogens list of the World Health Organization, existing in hospital patients and environment. Many clinical manifestations such as pneumonia, wound infection have posed huge disease burden with massive economic loss. The infection is associated with high morbidity and mortality rates. The burden of this hospital pathogen demands for attention especially with the surge in the resistant strains distribution. In the area of this study, distribution patterns have not extensively been study therefore, the research focused on the distribution pattern of hospital isolated *Acinetobacter baunmanni* from selected tertiary health facilities in Rivers State Nigeria. The observational study involved isolation and biochemical identification as well as molecular assay were performed using standardized methods was conducted in two main tertiary hospitals located in Port Harcourt metropolis, Rivers State, Nigeria. Statistics was performed for percentage and frequency distribution. Kruskal-wallis and Mann-WWitney test were used to compare difference in distribution at 0.05 level of significance. Statistical Package for Social Science version 21 was used for the statistics.368 samples from two institutions; RSUTH 185 (50.3%) and UPTH 183 (49.7%). Females 187 (50.8%) were more compared to the males 181(49.2%) with significant difference ($p<0.05$) in isolate distribution but no statistically significant difference ($p>0.05$) for location and sample. Also, sample distribution was uneven 59 (16.0%), 202 (54.9%), and 107 (29.1%) for aspirate, urine and wound respectively. 2.4% was recorded in the preliminary investigation while 75% positive rate was observed using molecular method. Evidence of the presence of *Acinetobacter baumannii* in the hospital was established. Also, *Acinetobacter baumanii* was not isolated from aspirate sample however, this is subject to further investigation with increased sample size. Female subjects had a higher rate. The information obtained here is essential to guide therapeutics and management of targeted clinical manifestation. Therefore, the study serves as surveillance of *A. baumannii* found in the selected region.

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Keywords: Distribution Pattern , Hospital, *Acinetobacter baunmanni*, Tertiary Health Facilities, Rivers State, Nigeria

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Introduction

The ubiquitous nature of *Acinetobacter baumannii* has made the Gram-negative, non-motile bacterium to be associated with hospital-acquired infection at the global level. With respect to antimicrobial resistance, it has been categorized among the most dangerous multiple drug-resistant (MDR) pathogens globally and enlisted in the "priority 1: critical" pathogens list of the World Health Organization, existing in both living and non living things including humans, animals, foods and the environment-shelves and hospital beddings and other materials.

Another study puts it that; *Acinetobacter baumannii* is one organism predominant in the healthcare facility and various units in the hospitals have been implicated to harbour the *Acinetobacter baumannii* hence, has obtained a worldwide notoriety as a important nosocomial pathogen due to its common attribution with drug resistance particularly multi-drug resistance in addition to hospital-based epidemics. *A. baumannii* accounted for 9.08% of the total clinical pathogenic isolates and became the third most gram-negative bacteria in the clinical bacteria in 2019. *A. baumannii* is associated with severe infection; raised morbidity and high mortality rates which has culminated to massive economic loss (Lin & Lan, 2014). The death rate of *A. baumannii* infection is 7.8% to 23% in hospital generally and 10% to 43% in Intensive care unit (Falagas et al., 2016).

There are varying distribution patterns of *Acinetobacter baumannii* which are based on several factors like geographic location, specific site sampled, and others. This has produced difference infection frequencies for prevalence and incidence rates. There is an extensive disparity in the incidence of *A. baumannii* infections between different countries including Nigeria. Nevertheless, its continuous transmission of *A. baumannii* is a concern of public health importance. For several years, the prevalence of infection has shown dramatically increase from 15.4% in 2004 to 48.5% in 2014 according to Rodloff & Dowzicky et al. (2017). Historically, *A. baumannii* emerged as a significant nosocomial pathogen and several epidemics were recorded with about 24% death rate Graser et al., 1993; Seifert et al., 1993; Seifert & Baginski (1992). Clinically, *A. Baumannii* causes pneumonia particularly ventilator-associated pneumonia in the hospital. Also, surgical wound infection, meningitis and other forms of clinical manifestations, principally in immune-compromised patients as previously documented (Graser et al., 2018; Antunes et al., 2014).

The burden of this hospital pathogen demands for attention especially with the surge in the resistant strains distribution. In the area of this study, distribution patterns have not

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extensively been study therefore, the research focused on the distribution pattern of hospital isolated *Acinetobacter baunmanni* from selected tertiary health facilities in Rivers State Nigeria. The information about A. *baumannii* distribution pattern is essential to guide therapeutics and management of targeted clinical manifestation. Therefore, the study serves as surveillance of *A. baumannii* found in the selected region.

Methodology

Port Harcourt metropolis in Rivers State, Nigeria was the area of the study. Port Harcourt has a tropical monsoon climate with lengthy and heavy rainy seasons and with very short dry seasons. The harmattan (dusty winds) which are prevalent in most part of the country are not as pronounced in Port Harcourt. The hottest and driest month in December and the heaviest rains fall in September. Temperature in Port Harcourt stay relatively constant tough and average about 25^{O}C to 28^{O}C. Port Harcourt is suited on on Latitude 4^{0} 46' 38"N longitude; 7^{0}00' 48". The occupation predorminate is farming both fish and crop farming. Industrial activities remain intense with oil and gas as the mainstay since its discovery. Study sites used were the two main tertiary hospitals which served as referral centres for the state and other neighbouring states.

Ethical approval was sought from the the Ethic Committee of the University of Port Harcourt Teaching Hospital and Rivers State Teaching Hospital and study participations were informed about the study, a questionnaire shaped and written informed consents were obtained from each of the participants before urine specimens were isolated from them.

Molecular procedure involved DNA extraction and the extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. Furthermore, the 16s rRNA region of the rRNA gene of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3'

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primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.5uM and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 52ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extention, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a blue light transilluminator.

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Results

The study evaluated the distribution pattern of Hospital Isolated *Acinetobacter baunmanni* isolated from selected tertiary health facilities in Rivers State Nigeria. The study was performed in a systematic order with an initial preliminary investigation using the conventional microbiological techniques of culture and biochemical assay. Thereafter, the molecular assays were performed based on the study objectives. The outcome of the findings from this study revealed the following; two main tertiary hospitals were the locations for this study namely; RSUTH 185 (50.3%) and UPTH 183 (49.7%). With respect to gender, females 187 (50.8%) were more compared to the males 181(49.2%). Based on sample type, the distribution was uneven 59 (16.0%), 202 (54.9%), and 107 (29.1%) for aspirate, urine and wound respectively.

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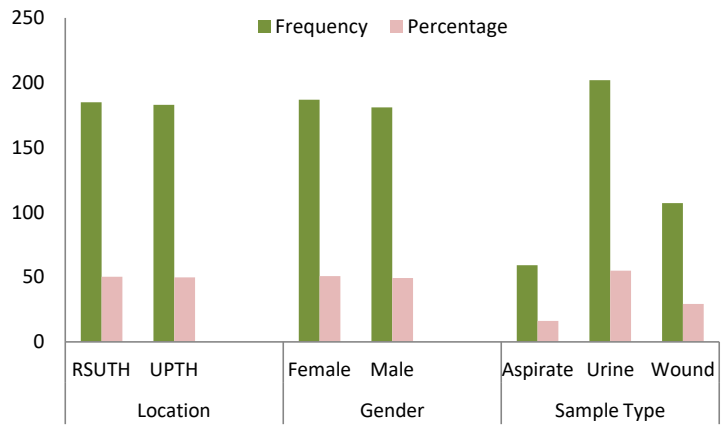

Figure 1: Column Chart showing Frequency Distribution of Location, Gender and Sample Type

The study performed a preliminary investigation prior to the molecular assay. The investigation revealed isolates of different types at varying rates. Table 4.2 presents the Frequency Distribution of Probable Isolates by Gender from the preliminary investigation. A total of 9 (2.4%) *Acinetobacter baumannii* were isolated, 6 (3.2%) females and 3 (1.7%) males in the preliminary investigation

Other microorganisms were isolated although these were not isolates of interest namely; *Enterobacter aerogenes, Enterobacter cloacae, Enterococcus faecalis, Klebsiella hormaechei, Klebsiella pneumonia, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus , Staphylococcus epidermidis, Streptococcus pneumoniae,* and *Streptococcus pyogenes. Staphylococcus aureus* was the highest microorganism isolated in this study with a total 78 (21.2%); 38 (20.3%)females and 40 (22.1%) males.

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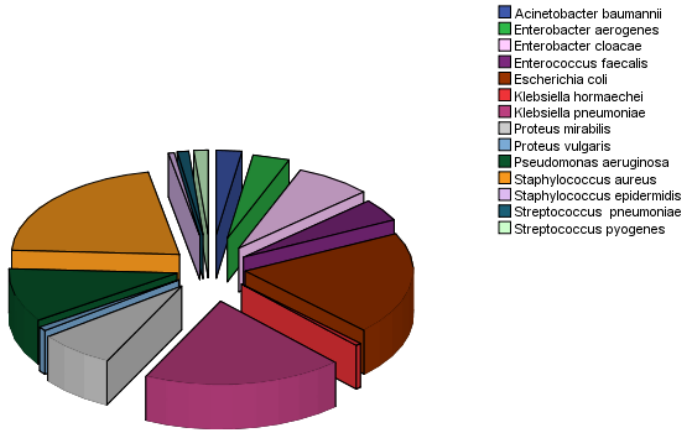

Figure 2: Pie Chart showing Distribution of *Acinetobacter baumannii* and other Isolates

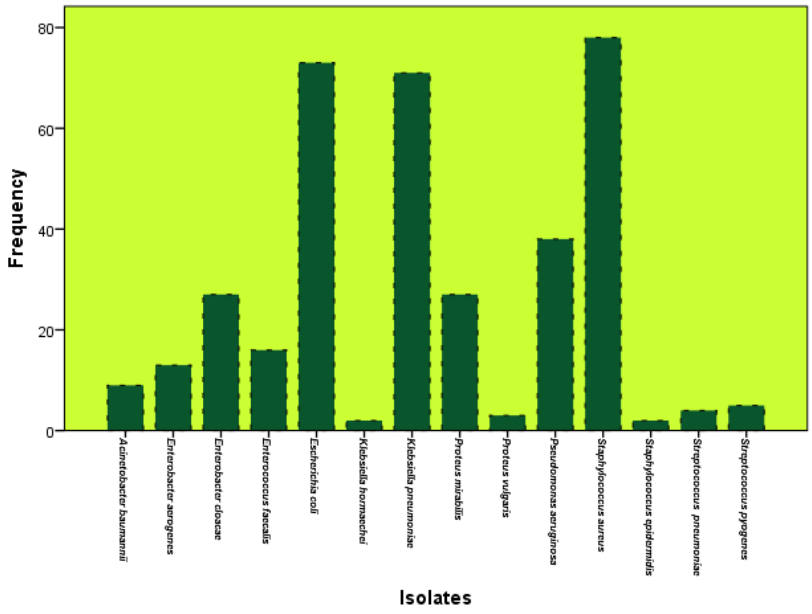

Figure 3.: Bar Chart of Probable Isolate Distribution based on Biochemical Technique (N=368)

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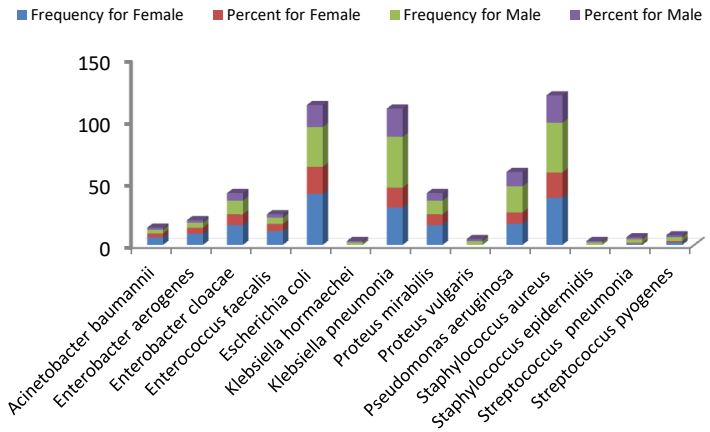

Figure 4: Frequency Distribution of Probable Isolates by Gender

Furthermore, Table 1 illustrated the Mann-Whitney U showing Comparative Analysis of Preliminary Distribution of Isolate by Gender. Result showed a statistically significant difference in the distribution of isolates obtained from male and females (Mann-Whitney U = 14485.500, p = 0.02). This implies that the microorganisms isolated from males and females in this study were dissimilar.

Table 1: Mann-Whitney U *showing Comparative Analysis of Preliminary Distribution of Isolate by Gender*

Gender	N	Mean Rank	Sum of Ranks	Mann-Whitney U	p-value	Remark
Female	187	171.46	32063.50	14485.500	0.02	Significant
Male	181	197.97	35832.50			
Total	368					

P<0.05=Sig=Significant, p>0.05=NS=Not Significant

Table 2: Frequency Distribution of Probable Isolates according to locations sampled. The finding demonstrates that *Klebsiella hormaechei* was the least sampled. 1 (0.5%) each for RSUTH, and UPTH. On the other hand, *Staphylococcus aureus* was the highest isolated in this study. 39(21.1%), and 39(21.3%) for RSUTH and UPTH correspondingly.

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Comparatively, RSUTH and UPTH shared similar as well as varying distribution of isolates as reported in this study. Besides, *Acinetobacter baumannii* isolated from RSUTH was 3 (1.6%) whereas, UPTH was 6 (3.3%), See table 2.

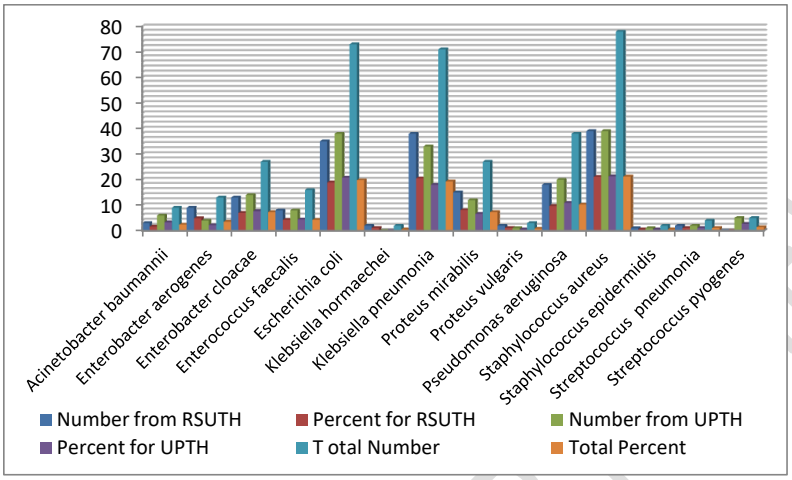

Figure 5: Frequency Distribution of Probable Isolates by Location

Table 3 shows the difference in the distribution of microorganisms isolated from RSUTH and UPTH locations. The analysis revealed null evidence of statistical significance (Mann-Whitney U, p=0.67). This suggests that, the microorganisms isolated from RSUTH and UPTH are the same in terms of species and rate of distribution.

Table 3: Mann-Whitney U *showing Comparative Analysis of Preliminary Distribution of Isolate by Location*

Location	N	Mean Rank	Sum of Ranks	Mann-Whitney U	p-value	Remark
RSUTH	185	182.16	33699.50	16494.500	0.67	NS
UPTH	183	186.87	34196.50			
Total	368					

P<0.05=Sig=Significant, p>0.05=NS=Not Significant

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Furthermore, figure 5 and 6 represent the Frequency Distribution of Probable Isolates based on the type of Sample the organisms were isolated from. The study outcome showed that urine and wound only had *Acinetobacter baumannii* at 7(3.5%) and 2 (1.9%) respectively; while *Acinetobacter baumannii was not isolated from* aspirate sample.

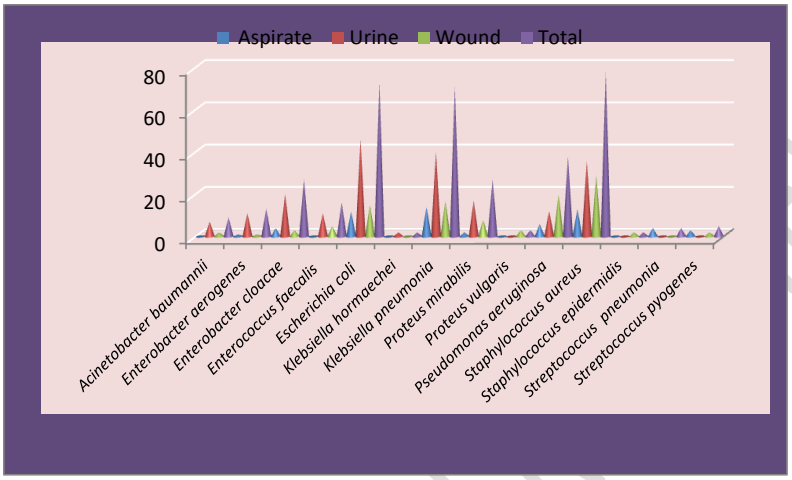

Figure 5: Frequency Distribution of Probable Isolates

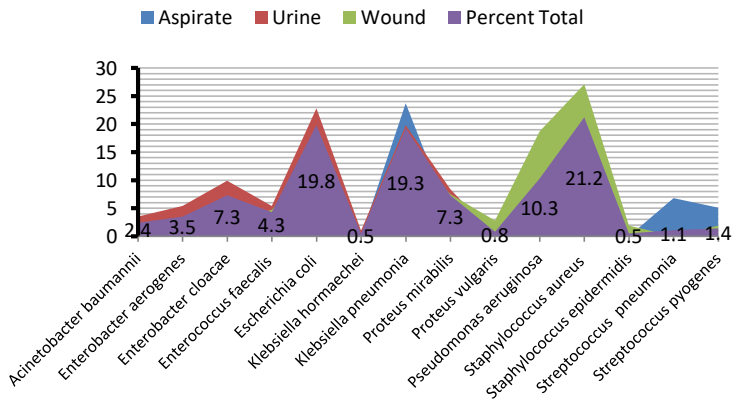

Figure 6: Percentage Distribution of Isolates by Sample

Table 4.: reports the Mann-Whitney U showing Comparative Analysis of Preliminary Distribution of Isolate by any two Samples. A significant discrepancy exist between Aspirate and Urine Isolates distribution (Mann-Whitney U = 4248.500, p =0.00). Similarly, distribution of isolates found in Urine and Wound proved indication of statistical disparity (Mann-Whitney U = 7296.500, p = 0.00). On the contrary, no statistically significant variation exists between Aspirate and Wound isolates distribution (Mann-Whitney U = 3095.000, p = 0.83).

Table 5.: Mann-Whitney U *showing Comparative Analysis of Preliminary Distribution of Isolate by Sample*

Sample Type	N	Mean Rank	Sum of Ranks	Mann-Whitney U	p-value	Remark
Aspirate and Urine Isolates						
Aspirate	59	159.99	9439.50	4248.500	0.00	Sig
Urine	202	122.53	24751.50			
Total	261					
Aspirate and Wound						
Aspirate	59	82.46	4865.00	3095.000	0.83	NS
Wound	107	84.07	8996.00			
Total						
Urine and Wound						
Urine	202	137.62	27799.50	7296.500	0.00	Sig
Wound	107	187.81	20095.50			
Total	309					

P<0.05=Sig=Significant, p>0.05=NS=Not Significant

Additionally, comparative analysis of all three distribution of isolates by sample type showed statistical significant variance using Kruskal-wallis (Chi Square = 27.281, df = 2, p =0.05). This means that the distribution of microorganisms isolated in this study across the three sample type are not equal. See table 6.

Table 6: Kruskal-wallis Test *showing Comparative Analysis of Preliminary Distribution of Isolate by Sample*

Sample Type	N	Mean Rank	Chi-Square	Df	p-value	Remark
Aspirate	59	212.45				
Urine	202	158.65				

UNDER PEER REVIEW

			27.281	2	0.00	Sig
Wound	107	217.88				
Total	368					

P<0.05=Sig=Significant, p>0.05=NS=Not Significant

Prevalence of *Acinetobacter baumannii* from Preliminary Study

Generally the overall *p*revalence of *Acinetobacter baumannii* from the Preliminary Study according to the culture and simple biochemical tests was 9 (2.4%). Gender specific prevalence revealed 6 (3.2%) and 3 (1.7%) for females and males accordingly. Based on location, the prevalence rate for RSUTH = 3(1.6%) and UPTH =6 (3.3%). Furthermore, with respect to the sample type, the specific prevalence obtained were 0 (0%%)7 (3.5%) and 2(1.8%) for aspirate, urine and wound respectively. See table 7 for detail.

Table 7; Prevalence of Acinetobacter baumannii

Variables	**Number Tested**	**Number Negative**	**Number Positive**	**Prevalence (%)**
Female	187	181	6	3.2
Male	181	178	3	1.7
Total	368	359	9	2.4
RSUTH	185	182	3	1.6
UPTH	183	177	6	3.3
Total	368	359	9	2.4
Aspirate	59	59	0	0
Urine	202	195	7	3.5
Wound	107	105	2	1.8
Total	368	359	9	2.4

Note: Prevalence of Acinetobacter baumannii obtained from preliminary investigation (biochemical) = 2.4%

Molecular Technique

UNDER PEER REVIEW

Table 8 displayed the frequency and percent distribution of variables used for molecular assay following the preliminary investigation. The two locations had equal number 6 (50%) each. Similarly, gender had equal distribution, 6 (50%) each for male and female. However, distribution of samples collected was skewed, no aspirate but urine and wound swab as 10 (83.3%) and 2 (16.7%) accordingly. Also, different isolates specifically three (3) species of microorgansisms were observed using the molecular technique namely: *Acinetobacter baumannii* 9 (75.0%), *Klebsiella hormaechei* 1 (8.3%), and *Klebsiella pneumonia* 2 (16.7%).

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Table 8: Frequency using Molecular Technique

Variable	Classification	Frequency	Percent
Location	RSUTH	6	50.0
	UPTH	6	50.0
	Total	12	100.0
Gender	Female	6	50.0
	Male	6	50.0
	Total	12	100.0
Sample	Urine	10	83.3
	Wound	2	16.7
	Total	12	100.0
Isolates	*Acinetobacter baumannii*	9	75.0
	Klebsiella hormaechei	1	8.3
	Klebsiella pneumonia	2	16.7
	Total	12	100.0

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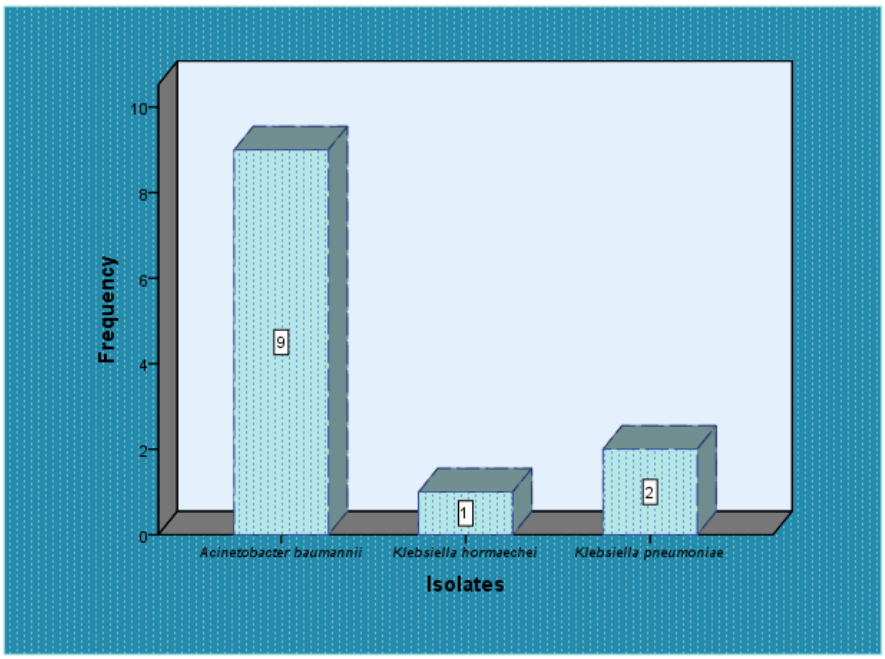

Bar Chartshowing Distribution of Isolates Obtained using Molecular Technique

Figure 7: Bar Chart of Isolate Distribution based on Molecular Technique (N=12)

Table 9 represents the distribution rate of *Acinetobacter baumannii as* obtained using Molecular Technique. A total of 12 samples were assayed and 9 reported positive for *Acinetobacter baumannii* and a rate of 75% was obtained as the overall distribution rate of *Acinetobacter baumannii* based on molecular method in this study. With regards to specific variables, location showed varying rates of 50% and 100% for RSUTH and UPTH respectively. Gender specific rate of *Acinetobacter baumannii* revealed 100% and 50% for female and male. While sample type observed 70% and 100% for urine and wound swab correspondingly.

Table 9:Distrbution Rate of *Acinetobacter baumannii* by Molecular Technique

Variables	Number Tested	Number Negative	Number Positive	Positive Rate (%)

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RSUTH	6	3	3	50.0
UPTH	6	0	6	100.0
Total	12	3	9	75.0
Female	6	0	6	100.0
Male	6	3	3	50.0
Total	12	3	9	75.0
Urine	10	3	7	70.0
Wound	2	0	2	100.0
Total	12	3	9	75.0

Discussion

The emergence of *A. baumannii* as a cause of nosocomial infection worldwide (Kais *et al.*, 2016; Muhammad *et al.*, 2018) is a challenging public health problem demanding attention particularly the antibiotic resistant strains. This gram negative organism possesses some features and arsenal typical for its function. Some of which are the presence of genetic materials such as resistance and virulence genes used during adverse condition. The presence of resistant and virulence genes remain key. In addition, the characteristics of the pathogen is crucial; Species of *Acinetobacter* are good in the formation of biofilm therefore known as one of the producing bacteria. This biofilm production aids the organisms in surviving adverse environmental conditions like that seen in the hospital environment **(**Ayenew *et al*., 2021).

The increasing rate of *Acinetobacter baumannii* in recent time as a major pathogen associated with hospital acquired 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).

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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. The rate of distribution, antibiogram pattern (including MDR and MARI), resistance, and virulence genes were evaluated using conventional biochemical and molecular methods sequentially.

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Distribution of *Acinetobacter baumanii*

Based on the overall distribution of *Acinetobacter baumanii* by biochemical test method as reported in this study, the rate 2.4% is lower compared to 4.6% recorded in Lagos and 14% reported in Ibadan. Similarly, lower than 9% observed in France, 14.5% reported by Kessaries and colleagues (2006). Furthermore, other studies differed with this study with higher rates of distribution such as 13.9%, 8.4%, and 3% for Lul *et al.* (2009), Oberoi *et al.* (2009) and Iregbu *et al.* (2002) respectively. Notably, different studies have shown diverse results with some similarities. The variations in these studies might be as a result of several things from time/period, geographical location including study area, site and population. Also, hugely on study design and methodology as well as assay methods, sample size and other factors.

Methodology has a role in identification because of different detection capacities. Following the molecular method, this study is in conformity with earlier study which recorded 79% rate as reported by Nwadike *et al.* (2014). This is equivalent to the positive rate of 75% obtained in this study using the molecular technique preceding the preliminary investigation with the biochemical tests. From the molecular assay, the study confirmed nine isolates as *A. Baumannii* out of twelve. This finding did not significantly differ from an earlier study which confirmed twelve isolates as *A. Baumannii* out of fourteen Isolates (Alkali *et al.*, 2019). However, the distribution rate of *A. Baumannii* based on the molecular method used varied

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with Namita *et al.* (2012) and Odewale *et al.* (2016). It is important to note that, the conventional biochemical identification might be limited and reliance on the result should be with caution or possible confirmation with higher technique like the molecular method. The distribution of A. Baumannii in this study is not inline with some related studies (Alkali *et al.*, 2019; Heydarpour *et al.*, 2017; Nwadike *et al.*, 2014).

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Distribution rate of *A. baumannii* obtained in this study according to gender which showed more females harbouring the organism compared to the male is in opposition with the work of Alkali *et al.* (2019) and Pal *et al*. (2017) which reported higher prevalence in male. The high rate of *A. baumannii* in female could be as a result of the female anatomical structure including the use of invasive procedures on the female patients. Nevertheless, this study is in opposition with previous work by Nwadike, Ojide, & Kalu (2014) which revealed the female preponderance observed was not statistically significant despite the difference in distribution but in this study the distribution was observed to be significant. Moreover, this study lacks support by the study of Victor *et al.* (2019) which reported that the male subjects harboured the isolates more.

Isolate distribution based on sample in this study differed from a recent report were wound was the main source of isolate and isolate was obtained from aspirate **(**Ayenew *et al.*, 2021). But in this study, urine had the highest number of isolates, followed by wound and aspirate had null isolate. Nwadike and colleagues' (2014) findings disagreed with this study in that, this study did not isolate *Acinetobacter baumanii* from aspirate whereas, theirs did even had majority isolated from aspirate specifically trachea. Equally, urine having the highest *A. baumannii* isolate is in consonance with earlier study (Alkali *et al.*, 2019). Correspondingly, finding of Zuhair (2011) confirmed highest rate in urine. On the other hand, Pal *et al.* (2017)

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reported other sample sources such as suction tips and secretion to have high rate of *A. baumannii* isolates. Also, Victor *et al*. (2019) isolated *A. baumannii* from aspirate in a study.

Acinetobacter baumanii have been implicated to cause different types of nosocomial infections in man such as urinary tract infections, respiratory tract infections and septicemia according to Patwardhan *et al.* (2008). *Acinetobacter baumanii* survive even in non living things with high level of antibiotic resistant and vast transmission as described by Oberoi *et al.* (2009) and Muhammad *et al.* (2018) at different times.

Conclusion/ Recommendation

The burden of hospital acquired *Acinetobacter baumanii* obtained in this study proved a degree of predominance with varying distribution patterns according to location and sample type investigated. *Acinetobacter baumanii* was not isolated from aspirate sample however, this is subject to further investigation with increased sample size. Female subjects had a higher rate. The outcome of this present study on the distribution patterns of hospital isolated *Acinetobacter baunmanni* from selected tertiary health facilities in Rivers State Nigeria have provided information and this serves as surveillance of *A. baumannii* found in the region of this study.

Based on the pragmatic evidence from this study, there is need for caution and intense bacterial particularly *Acinetobacter baumanii* surveillance including review of treatment guidelines because some of the isolates might probably be harbouring virulent and resistance genes. Although this is beyond the scope of this present study, further large scale studies can look in this direction.

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Conflict of Interest

None exists between authors