

Distribution Pattern of Hospital Isolated *Acinetobacter baumannii* 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, and wound infection have posed a huge disease burden with massive economic loss. The infection is associated with high morbidity and mortality rates. The burden of this hospital pathogen demands attention, especially with the surge in the resistant strains distribution. In the area of this study, distribution patterns have not extensively been studied therefore, the research focused on the distribution pattern of hospital isolated *Acinetobacter baumannii* from selected tertiary health facilities in Rivers State Nigeria. The observational study involved isolation and biochemical identification, as well as molecular assay, which was performed using standardized methods, was conducted in two main tertiary hospitals located in Port Harcourt metropolis, Rivers State, Nigeria. Statistics were performed for percentage and frequency distribution. Kruskal-Wallis and Mann-Whitney tests were used to compare the difference in distribution at a 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 a 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 a 75% positive rate was observed using the molecular method. Evidence of the presence of *Acinetobacter baumannii* in the hospital was established. Also, *Acinetobacter baumannii* was not isolated from the aspirate sample however, this is subject to further investigation with an increased sample size. Female subjects had a higher rate. The information obtained here is essential to guide therapeutics and the management of targeted clinical manifestation. Therefore, the study serves as surveillance of *A. baumannii* found in the selected region.

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

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 nonliving 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 worldwide notoriety as an 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 in 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 units (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 different infection frequencies for prevalence and incidence rates. There is an extensive disparity in the incidence of *A. baumannii* infections in 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 a dramatic 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 a 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 attention, especially with the surge in the resistant strains distribution. In the area of this study, distribution patterns have not

extensively been studied therefore, the research focused on the distribution pattern of hospital isolated *Acinetobacter baumannii* 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

The method adopted was the molecular method which includes DNA boiling, extraction, quantification, and amplification of DNA. 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 of the country is not as pronounced in Port Harcourt. The hottest and driest month is December and the heaviest rains fall in September. The temperature in Port Harcourt stays relatively constant though and averages about 25^oC to 28^oC. Port Harcourt is situated on Latitude 4^o 46' 38"N longitude; 7^o00' 48". The occupation predominant is farming both fish and crop farming. Industrial activities remain intense with oil and gas as the mainstay since its discovery. The study sites used were the two main tertiary hospitals which served as referral centers for the state and other neighboring states.

The 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 was amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on an 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 a template. The PCR conditions were as follows: Initial denaturation, 95°C for 5 minutes; denaturation, 95°C for 30 seconds; annealing, 52°C for 30 seconds; extension, 72°C for 30 seconds for 35 cycles and final extension, 72°C for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a blue light transilluminator.

Results

The study evaluated the distribution pattern of Hospital Isolated *Acinetobacter baumannii* 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.

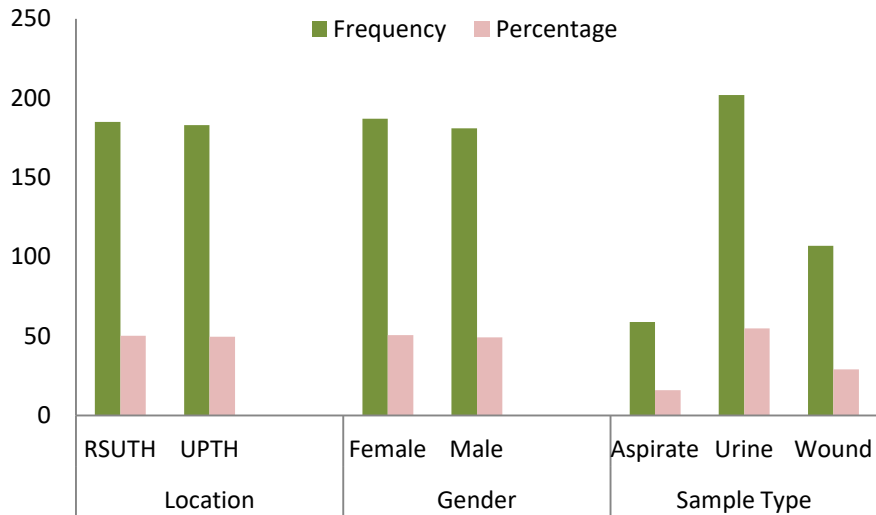


Figure 1: Column Chart showing Frequency Distribution of Location, Gender, and Sample Type

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 pneumonia*, and *Streptococcus pyogenes*. *Staphylococcus aureus* was the highest microorganism isolated in this study with a total of 78 (21.2%); 38 (20.3%) females and 40 (22.1%) males.

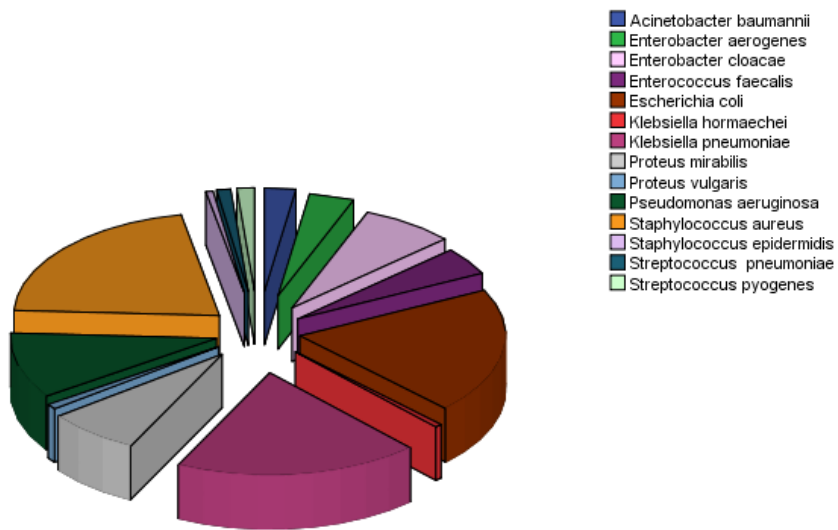


Figure 2: Pie Chart showing Distribution of *Acinetobacter baumannii* and other Isolates

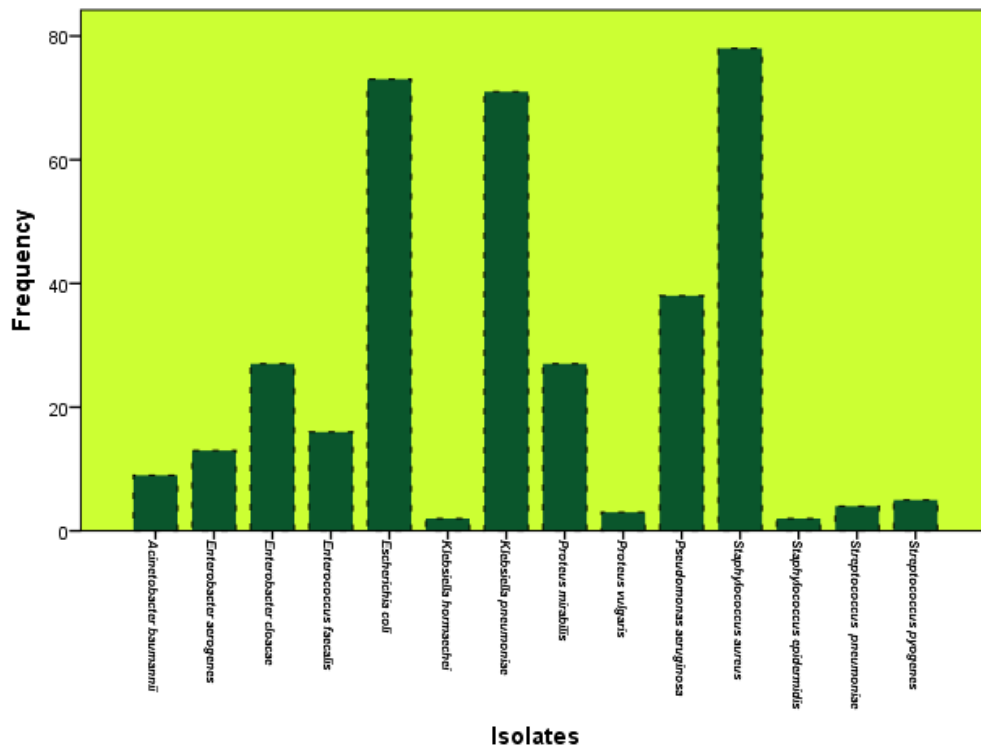


Figure 3.: Bar Chart of Probable Isolate Distribution based on Biochemical Technique (N=368)

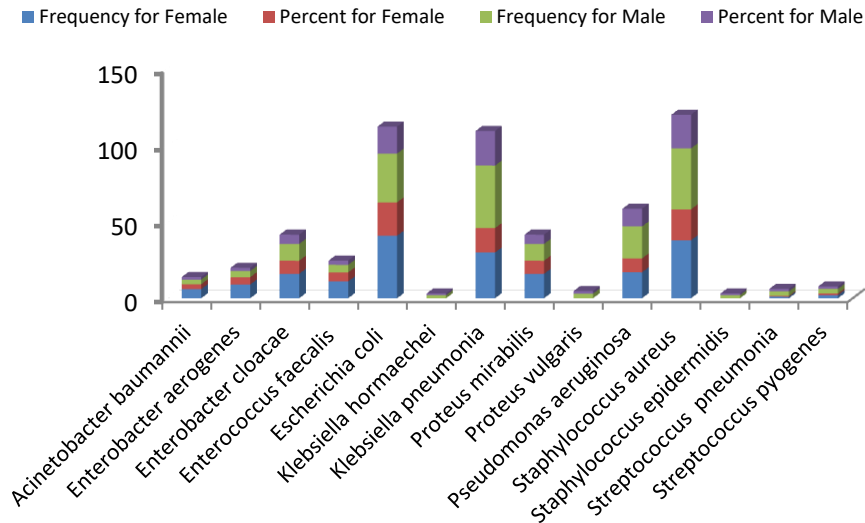


Figure 4: Frequency Distribution of Probable Isolates by Gender

Furthermore, Table 1 illustrated the Mann-Whitney U showing Comparative Analysis of Preliminary Distribution of Isolate by Gender. The result showed a statistically significant difference in the distribution of isolates obtained from males 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					

$P < 0.05 = \text{Sig} = \text{Significant}$, $p > 0.05 = \text{NS} = \text{Not Significant}$

The findings in Figure 4 demonstrate 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. 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.

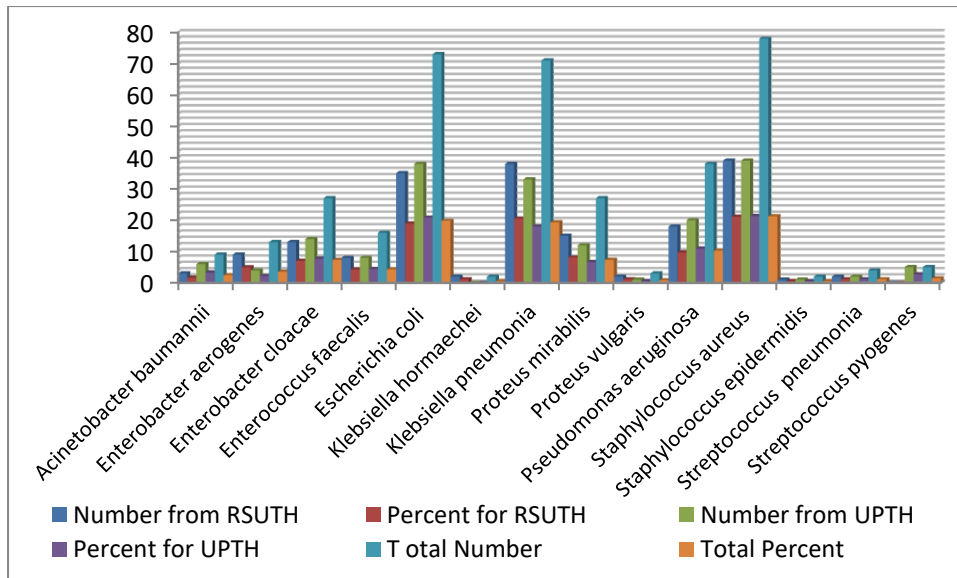


Figure 5: Frequency Distribution of Probable Isolates by Location

Table 2 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 2: Mann-Whitney U showing Comparative Analysis of Preliminary Distribution of Isolate by Location

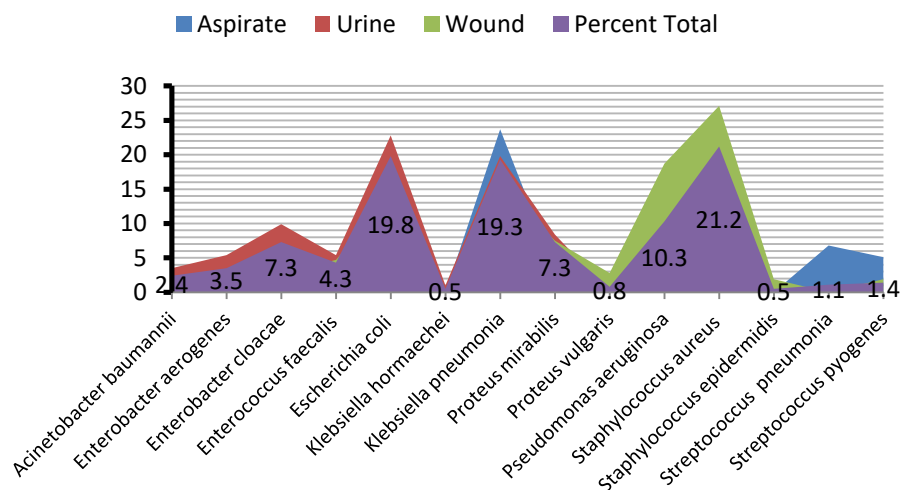


Figure 6: Percentage Distribution of Isolates by Sample

Table 3.: reports the Mann-Whitney U showing Comparative Analysis of Preliminary Distribution of Isolate by any two Samples. A significant discrepancy exists between Aspirate and Urine Isolates distribution (Mann-Whitney U = 4248.500, p =0.00). Similarly, the distribution of isolates found in Urine and Wound proved an 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 3.: Mann-Whitney U showing Comparative Analysis of Preliminary Distribution of Isolate by two 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					

$P < 0.05 = \text{Sig} = \text{Significant}$, $p > 0.05 = \text{NS} = \text{Not Significant}$

Additionally, a comparative analysis of all three distributions of isolates by sample type showed statistically 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 types is not equal. See table 4.

Table 4: 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	27.281	2	0.00	Sig
Wound	107	217.88				
Total	368					

$P < 0.05 = \text{Sig} = \text{Significant}$, $p > 0.05 = \text{NS} = \text{Not Significant}$

Prevalence of *Acinetobacter baumannii* from Preliminary Study

Generally, the overall prevalence of *Acinetobacter baumannii* from the Preliminary Study according to the culture and simple biochemical tests were 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 5 for detail.

Table 5; 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%

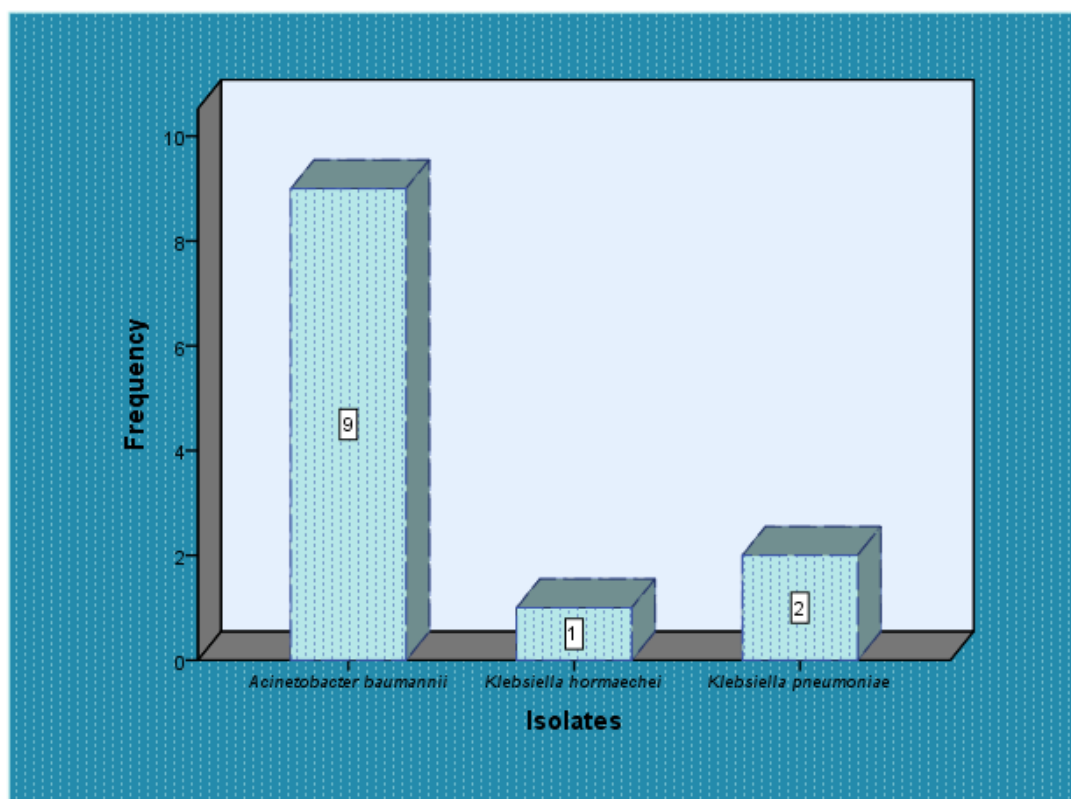
Molecular Technique

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

Table 6: 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	<i>Acinetobacter baumannii</i>	9	75.0
	<i>Klebsiella hormaechei</i>	1	8.3
	<i>Klebsiella pneumonia</i>	2	16.7
	Total	12	100.0



Bar Chart showing Distribution of Isolates Obtained using Molecular Technique

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

Table 7 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 the 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* was revealed at 100% and 50%

for females and males. While sample types observed were 70% and 100% for urine and wound swabs correspondingly.

Table 7 : Distrbution Rate of *Acinetobacter baumannii* by Molecular Technique

Variables	Number Tested	Number Negative	Number Positive	Positive Rate (%)
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 (Partwardhan et al., 2008; 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 conditions. The presence of resistant and virulence genes remains key. In addition, the characteristics of the pathogen are 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 times as a major pathogen associated with hospital-acquired infections is burdensome and this has resulted in 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 (Nwadike *et al.*, 2014) although, some have measured the burden with estimates of 35% - 45% with a mortality rate of 26% (Muhammad *et al.*, 2018; Xie *et al.*, 2018).

The study was a cross-sectional hospital-based study that investigated the phylogeny (evolutionary relationship) and antibiogram of *Acinetobacter baumannii* 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.

Distribution of *Acinetobacter baumannii*

Based on the overall distribution of *Acinetobacter baumannii* 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% was observed in France, 14.5% reported by Kessaries and colleagues (2006). Furthermore, other studies differed from 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 a result of several things from time/period, and geographical location including the study area, site, and population. Also, hugely on study design and methodology as well as assay methods, sample size, and other factors.

The methodology has a role in identification because of different detection capacities. Following the molecular method, this study conforms with an earlier study which recorded a

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 (Alkalin *et al.*, 2015). However, the distribution rate of *A. Baumannii* based on the molecular method used varied with Mushtaq *et al.* (2013) and Altun *et al.* (2014). 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 a higher technique like the molecular method. The distribution of *A. Baumannii* in this study is not inline with some related studies (Alkalin *et al.*, 2015 Nwadike *et al.*, 2014).

The distribution rate of *A. baumannii* obtained in this study according to gender which showed more females harboring the organism compared to the male is in opposition with the work of Alkali *et al.* (2019) and Awad *et al.* (2015) which reported higher prevalence in male. The high rate of *A. baumannii* in females could be a result of the female anatomical structure including the use of invasive procedures on the female patients. Nevertheless, this study is in opposition to 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 from the study of Victor *et al.* (2019) who reported that the male subjects harbored the isolates more.

Isolate distribution based on the sample in this study differed from a recent report where the 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 baumannii* from aspirates whereas, did even had the majority isolated from aspirates specifically trachea. Equally, urine having the highest *A. baumannii* isolate is in consonance with an earlier study (Altun , 2014). Correspondingly, the finding of Guckan *et al.*, (2015) confirmed the highest rate in urine. On the other hand, Mushtaq *et al.* (2013) reported other sample sources such as suction tips and secretion to have a high rate of *A. baumannii* isolates. Also, Victor *et al.* (2019) isolated *A. baumannii* from aspirate in a study.

Acinetobacter baumannii have been implicated to cause different types of nosocomial infections in men such as urinary tract infections, respiratory tract infections, and septicemia according to Alkalin *et al.* (2015). *Acinetobacter baumannii* survive even in non-living things with a high level of antibiotic resistance and vast transmission as described by Oberoi *et al.* (2009) and Muhammad *et al.* (2018) at different times.

Conclusion and Recommendation

The burden of hospital-acquired *Acinetobacter baumannii* obtained in this study proved a degree of predominance with varying distribution patterns according to the location and sample type investigated. *Acinetobacter baumannii* was not isolated from the aspirate sample however, this is subject to further investigation with an increased sample size. Female subjects had a higher rate. The outcome of this present study on the distribution patterns of hospital-isolated *Acinetobacter baumannii* from selected tertiary health facilities in Rivers State Nigeria has 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 a need for caution and intense bacterial particularly *Acinetobacter baumannii* surveillance including a review of treatment guidelines because some of the isolates might probably be harboring virulent and resistant genes. Although this is beyond the scope of this present study, further large-scale studies can look in this direction.

Acknowledgement

All tertiary health facilities and unit heads are well acknowledge for their massive supports.

Conflict of Interest

None exists between authors

Ethical Approval and Consent:

Ethical approval was sought from 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 consent were obtained from each of the participants before urine specimens were isolated from them

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