Bacteriological Profile and Multidrug Resistance Patterns of Isolates from Sputum of Adults with Community Acquired Pneumonia in Diobu, Port Harcourt, Nigeria: A Retrospective Study

Abstract

Community acquired pneumonia is a major global public health concern given its substantial contribution to the mortality and morbidity associated with infectious diseases, the huge losses in human and economic resources and the increasing challenges in treatment due to multidrug resistance (MDR). This retrospective cross sectional descriptive study reviewed laboratory records of adults clinically diagnosed with community acquired pneumonia (CAP) between January 1, 2017, and December 31, 2022. A total of 308 sputum specimens meeting the inclusion criteria were reviewed and yielded 135 (43.8%) bacterial strains. The 135 bacterial strains spread across 9 species had *Streptococcus pneumoniae* as the dominant species with 41(30.4%); followed by *Staphylococcus aureus* 21(15.6%), *Escherichia coli* 16(11.9%), *Klebsiella pneumoniae* 16(11.9%), *Pseudomonas aeruginosa* 12(8.9%), *Streptococcus pyogenes* 12(8.9%), *Proteus mirabilis* 10(7.4%), *Enterobacter cloacae* 5(3.7%) and *Acinetobacter baumannii* 2(1.5%). The cumulative resistance profile was 45.7%; the most resistant bacterial specie was *Pseudomonas aeruginosa* with a resistance profile of 50%; followed by*Staphylococcus aureus* (48.6%), *Enterobacter cloacae* (48.0%), *Streptococcus pyogenes* 47.5%, *Streptococcus pneumoniae* 47.3%, *Klebsiella pneumoniae* 45.6%, *Proteus mirabilis* 42.0%, *Escherichia coli* 40.0% and *Acinetobacter baumannii* 35.0%. The MDR prevalence was 85.9% including 36.3% extensively resistant strains; but no pan-drug resistant trains; while 14.1% were non-multidrug resistant. This study has contributed to the data on bacteriological profile and antimicrobial resistance patterns in aetiological agents of community acquired pneumonia in Port Harcourt. The high prevalence of drug resistance implies that many people are likely to be infected, while most of the antibiotics are losing potency against the bacterial pathogens. It is advised that regulatory laws on drug control be revised as it pertains antibiotics, the regulatory agencies should be compelled to perform their statutory duties while there is need to sensitize the populace on the dangers of antibiotic abuse and misuse.

Key words:*Community Acquired Pneumonia, Public Health, Multidrug Resistance, Antimicrobial Resistance, Streptococcus pneumoniae, Klebsiella pneumoniae.*

Introduction

Pneumonia connotes respiratory tract infections involving the lungs, typically affecting the alveoli and distal airways; and caused by a variety of microorganisms, notably bacteria, viruses and fungi[1,2]. In contrast to Hospital acquired pneumonia (HAP) which is defined as that acquired after a minimum of 48 hours stay in the hospital, Community acquired pneumonia refers to that contracted outside the hospital setting or before 48 hours of stay in the hospital [3]. It is a leading cause of sepsis [4], accounting for an ample portion of the global infectious diseases burden, mortality and morbidity; especially in resources challenged countries [5]. The World Health Organization (WHO) estimates the annual global mortality arising from Community acquired pneumonia at between three and four million [6].

Community acquired pneumonia poses a major global public health concern given its substantial contribution to the population of persons visiting hospitals for the treatment for infectious diseases, the resultant huge losses in human and economic resources, the degree of associated morbidity and mortality and the increasing challenges in treatment due to multidrug

resistance to commonly used antimicrobial agents. A huge chunk of the disease burden is borne by resource challenged developing countries like Nigeria where the disease is responsible for 2.5% to 5.7% of hospital admissions, with a morbidity prevalence of between 7.4% and 26% for hospitalized persons and constituting 15.3 to 24.9% of persons admitted to hospital for respiratory tract infections[5,6,7]. The global incidence of CAP has been reported at 1.5 to 14 cases per 1000 person-years, dependent on a number of variables such as economic levels, geographical location, climatic factors and socio-demographics [8].

Viruses constitute a dominant portion of the aetiologic agents of CAP, followed by bacteria and fungi. Bacterial isolates associated with the etiology of the disease include culturable and non-culturable bacteria. The culturable bacteria include *Streptococcus pneumoniae, Streptococcus pyogenes Staphylococcus aureus, Escherichia coli, Klebsiella species, Moraxella catarrhalis Hemophilus influenzae, Pseudomonas* species Citrobacter species, Enterococcus species, Enterobacter species, Proteus species, Nocardia and *Acinetobacter* species; while the non-culturable bacteria include *Legionella pneumophila, Mycoplasma pneumoniae, Chlamydophila pneumoniae,* and *C. psittaci*; fungi include *Candida* species [3,9,10].

A number of factors are attributable with increased incidence of infection with CAP, severity of the disease and rate of morbidity. These include old age, poor nutritional status, infancy, presence of comorbidities, smoking, alcohol abuse, abuse of antibiotics, corticosteroids etc. [3]. Some of the co-morbidities include human immunodeficiency virus (HIV) Infection, chronic obstructive pulmonary disease (COPD), diabetes mellitus, structural lung disease and congestive heart failure [3,6]. There are also possible variations between and within regions, countries, localities and seasonally[3].

A palpable difficulty in the treatment of CAP is that posed by the high and increasing global problem of antimicrobial resistance, which is disproportionately heavy on the resource challenged countries such as Nigeria, characterized by high burden of infectious diseases, antibiotics abuse and misuse due to defective of non-existent regulation, poor quality drugs, inaccessibility of the few quality drugs due to high costs amidst pervasive poverty [7]. Some of the bacteria associated with CAP are part of the ESKAPE pathogens (*Enterococcus* spp., *Staphylococcus aureus Klebsiella pneumoniae Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp) notorious for being highly resistant to many antimicrobial agents[11,12].

There‘s paucity of published data on bacteriological profile and antimicrobial resistance patterns in aetiological agents community acquired pneumonia in Nigeria, particularly in Port Harcourt. This study is thus aimed at identifying the bacterial agents causing community acquired pneumonia in Port Harcourt and their antimicrobial resistance patterns.

Materials and Methods

Study design, period, and setting.

In this retrospective cross sectional descriptive study, laboratory records of microscopy, culture and susceptibility analysis of sputum specimens carried out at Diagnostix and Scientifique

Research Laboratories, Port Harcourt, Nigeria were reviewed. The analysis involved adult patients from public and private healthcare facilities with presumptive diagnosis of CAP between January 1, 2017, and December 31, 2022. The specimens included in the study were those with complete records of the age and sex of subjects, isolated organisms, resistant and susceptible antimicrobial drugs.

Records of Isolation and Identification of Organisms

As contained in the standard operating procedure (SOP), the HVS specimens were cultured on blood agar and MacConkey agar (Oxoid, Hampshire, England); then incubated under aerobic conditions at 37 °C for 18 to 24 hours. The culture plates were examined visually for growths and the colonial morphologies were recorded; followed by gram-staining and biochemical testing. The morphological, biochemical, and physiological data were inputted into the ABIS online bacterial identification software, and the organisms were identified by the best match [13,14,15, 16].

Antimicrobial Susceptibility Testing

Antimicrobial Susceptibility Testing, (as stated in the SOP) was performed on the bacterial isolates by employing the Kirby Bauer disk diffusion method using Mueller-Hinton agar (Oxoid, Hampshire, England)[17]. The following antimicrobial agents tested: Ampicillin/ cloxacillin(20µg), Azithromycin (30 µg), Ceftriaxone (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (10 µg), Levofloxacin(20µg), Gentamicin (10 µg), Norfloxacin (10 µg), Rifampicin (20 µg), Streptomycin (30 µg) (Oxoid, England) Resistance data were read and interpreted in accordance with the standards of the Clinical Laboratory Standards Institute (CLSI)[11,17]

Data Analysis

The collected data were examined manually with excel spreadsheet for completeness clarity and consistencies then aligned and edited as appropriate before transferring to GraphPad 8.0.2 for the analysis.

Results

Microbial cultures of 308 sputum specimens obtained from persons with presumptive diagnosesof Community acquired Pneumonia yielded 135 (43.8%) bacterial strains from a total of 126 (40.9%) positive cultures, including 9 (2.9%)mixed bacterial growths. The specimens were obtained from 182 (59.1%) females and 126 (40.9%) males. The female specimens yielded 77(42.3%) bacterial strains from 71(39.0%) positive cultures which include 6 (3.3) mixed bacteria colonies; while the specimens obtained from males produced 58 (46.0%) bacterial strains from 55(43.7%) which include 3 (2.4) mixed bacterial colonies. The age brackets of 41-50 and 60 and above with the highest and least numbers of samples of 67 ((21.8%) and 53

(17.2%) respectively, yielded the highest number of isolates with 29 strains apiece. The least number of strains were obtained from the 18 – 30 and 51 – 60 age brackets with 25 each. (Table 1)

Age Brackets	All Samples				Female Samples				Male Samples			
	SS(%)	TBG (%)	MBG (%)	NBS (%)	SS (%)	TBG (%)	MBG (%)	NBS (%)	SS (%)	TBG (%)	MBG (%)	NBS (%)
18 -30	59	22	3	25	35	12	2	14	24	10	1	11

Table 1: Distribution of Sputum Samples and bacterial Growths from the Sputum of Adults with Community Acquired Pneumonia in Diobu, Port Harcourt, Nigeria

	(19.2)	(37.3)	(5.1)	(42.4)	(19.2)	(34.3)	(5.7)	(40.0)	(19.1)	(41.7)	(4.2)	(45.8)
31 – 40	63 (20.5)	27 (42.9)	0	27 (42.9)	36 (19.9)	15 (41.7)	0	15 (41.7)	27 (21.4)	12 (44.4)	0	12 (44.4)
41 – 50	67 (21.8)	27 (40.3)	2 (3.0)	29 (43.3)	38 (20.9)	14 (36.8)	1 (2.6)	15 (39.5)	29 (23.0)	13 (44.8)	1 (3.4)	14 (48.3)
51 – 60	66 (21.4)	24 (36.4)	1 (1.5)	25 (37.9)	40 (22.0)	15 (37.5)	0	15 (37.5)	26 (20.6)	9 (34.6)	1 (3.9)	10 (38.5)
61 and above	53 (17.2)	26 (49.1)	3 (5.7)	29 (54.7)	33 (18.1)	15 (45.5)	3 (9.1)	18 (54.5)	20 (15.9)	11 (55.0)	0	11 (55.0)
Total	**308 (100)**	**126 (40.9)**	**9 (2.9)**	**135 (43.8)**	**182 (59.1)**	**71 (39.0)**	**6 (3.3)**	**77 (42.3)**	**126 (40.9)**	**55 (43.7)**	**3 (2.4)**	**58 (46.0)**

SS: Sputum samples; **TBG:** Total bacterial growths; **MBG:** Mixed bacterial growths; **NBS:** Number of bacterial strains

The 135 bacterial strains isolated from the sputum samples include 9 species belonging to 8 genera, with *Streptococcus pneumoniae* as the dominant species with 41 (30.4%); followed by *Staphylococcus aureus* 21 (15.6%), *Escherichia coli* 16 (11.9%), *Klebsiella pneumoniae* 16 (11.9%), *Pseudomonas aeruginosa* 12 (8.9%), *Streptococcus pyogenes* 12(8.9%), *Proteus mirabilis* 10(7.4%), *Enterobacter cloacae* 5 (3.7%) and *Acinetobacter baumannii* 2 (1.5%). The female samples contributed 77(57.0%) of the isolates while the males were 58 (43.0%) (Table 2) The Gram-positive bacteria were predominant with 54.8%, while gram-negative bacteria were 45.2%. (Figure 1)

The descriptive statistics indicate that the female samples have a range of 20.00 with minimum and maximum values at 2.000 and 22.00; the range for the male samples was 19, with minimum value of 0.00 and maximum value 19.00, while the total samples have a range of 39, minimum value of 2.00 and maximum value 41. The mean, standard deviation and standard error of mean

for the female samples were 8.556, 6.085 and 2.028, respectively; for the male samples, the values were 6.444, 5.503 and 1.834 respectively, while the same values for the total samples were 15.00, 11.32 and 3.775 respectively.

Table 2: Distribution of Bacterial Species obtained from Sputum of Adults with Community Acquired Pneumonia in Diobu, Port Harcourt, Nigeria

Bacterial Species	Females	Percent	Males	Percent	Total	Percent
Streptococcus pneumoniae	22	53.7	19	46.3	41	30.4
Staphylococcus aureus	13	61.9	8	38.1	21	15.6
Escherichia coli	10	62.5	6	37.5	16	11.9
Klebsiella pneumoniae	9	56.3	7	43.7	16	11.9
Pseudomonas aeruginosa	7	58.3	5	41.7	12	8.9
Streptococcus pyogenes	4	33.3	8	66.7	12	8.9
Proteus mirabilis	6	60.0	4	40.0	10	7.4
Enterobacter cloacae	4	80.0	1	20.0	5	3.7
Acinetobacter baumannii	2	100	0	0	2	1.5
Total	77	57.0	58	43.0	135	100

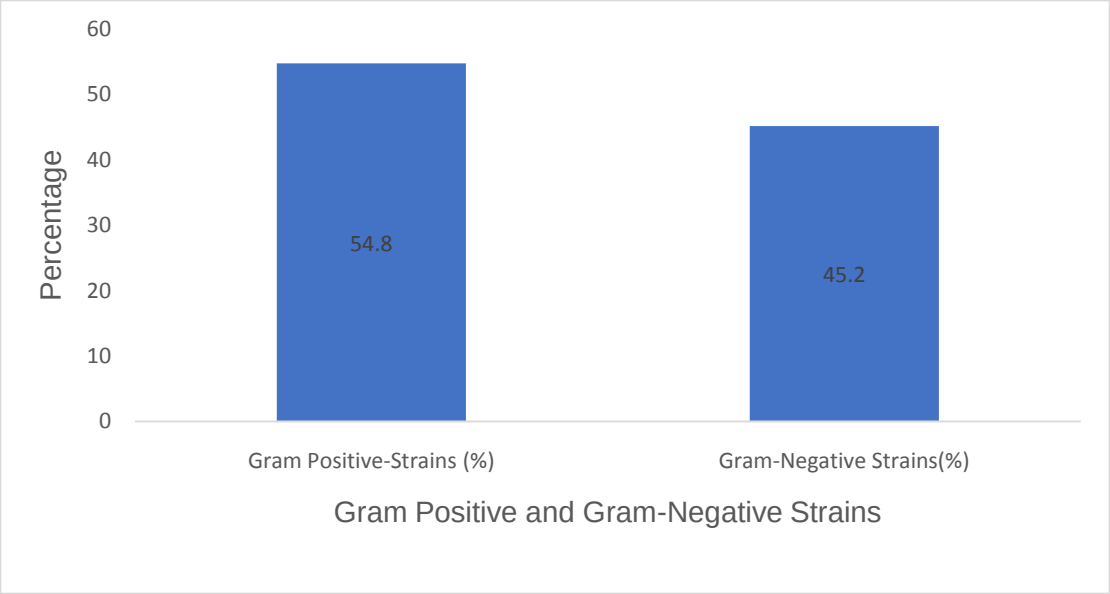

Figure 1: The percentages of Gram positive and Gram-negative Bacterial Isolates obtained from the Sputum of Adults with Community Acquired Pneumonia in Diobu, Port Harcourt, Nigeria

The cumulative resistance profile of the 135 bacterial strains isolated from the sputum of CAP patients against the 10 test antimicrobial agents was 45.7%; the most resistant bacterial specie was *Pseudomonas aeruginosa* with a resistance profile of 50%; followed by*Staphylococcus aureus* (48.6%), *Enterobacter cloacae* (48.0%), *Streptococcus pyogenes* 47.5%, *Streptococcus pneumoniae* 47.3%, *Klebsiella pneumoniae* 45.6%, *Proteus mirabilis* 42.0%, *Escherichia coli* 40.0% and *Acinetobacter baumannii* 35.0%. The antimicrobial agent against which most organisms were resistant was the first-generation fluoroquinolone norfloxacin against which 79.9% of the strains were resistant, followed by chloramphenicol (68.9%), rifampicin (65.2%), Ampicillin/cloxacillin (64.4%), streptomycin (41.5%), Azithromycin (40.0%), ceftriaxone (35.6%), Gentamicin (29.6%), ciprofloxacin (22.2%) and levofloxacin (21.5%). (Table 3)

Table 3: Antimicrobial Resistance Patterns in Bacterial Species obtained from the Sputum of Adults with Community Acquired Pneumonia in Diobu, Port Harcourt, Nigeria

Bacterial Isolates	**nx 10**	**APX**	**AZT**	**CTX**	**CHL**	**CPX**	**LV**	**CN**	**NB**	**RD**	**STR**	**Total**
Pseudomonas aeruginosa	120	9 (75.0)	5 (41.7)	3 (25.0)	10 (83.3)	3 (25.0)	3 (25.0)	4 (16.7)	9 (75.0)	8 (66.7)	6 (50.0)	60 (50.0)
Staphylococcus aureus	210	14 (66.7)	10 47.6)	9 (42.9)	15 (71.4)	6 (28.6)	5 (23.8)	7 (33.3)	14 (66.7)	13 (61.9)	9 (42.9)	96 (48.6)
Enterobacter cloacae	50	4 (80.0)	3 (60.0)	2 (40.0)	4 (80.0)	0	0	2 (40.0)	4 (80.0)	3 (60.0)	2 (40.0)	24 (48.0)
Streptococcus pyogenes	120	8 (66.7)	6 (50.0)	5 (41.7)	8 (66.7)	2 (16.7)	2 (16.7)	3 (25.0)	9 (75.0)	9 (75.0)	5 (41.7)	57 (47.5)
Streptococcus pneumoniae	410	26 (63.4)	15 (36.6)	17 (41.5)	25 (61.0)	12 (29.3)	9 (22.0)	14 (34.5)	30 (73.2)	28 (68.3)	18 (43.9)	194 (47.3)
Klebsiella pneumoniae	160	10 (62.5)	6 (37.5)	6 (37.5)	12 (75.0)	2 (12.5)	3 (18.8)	2 (12.5)	12 (75.0)	12 (75.0)	8 (50.0)	73 (45.6)
Proteus mirabilis	100	6 (60.0)	4 (40.0)	2 (20.0)	5 (50.0)	2 (20.0)	3 (30.0)	4 (40.0)	8 (80.0)	5 (50.0)	3 (30.0)	42 (42.0)
Escherichia coli	160	8 (50.0)	4 (25.0)	5 (31.3)	12 (75.0)	3 (18, 8)	4 (25.0)	4 (25.0)	10 (62.5)	10 (62.5)	4 (25.0)	64 (40.0)
Acinetobacter baumannii	20	2 (100)	1 (50.0)	1 (50.0)	2 (100)	0	0	0	1 (50.0)	0	0	7 (35.0)
Total	**1350 (100)**	**87 (64.4)**	**54 (40.0)**	**48 (35.6)**	**93 (68.9)**	**30 (22.2)**	**29 (21.5)**	**40 (29.6)**	**97 (79.9)**	**88 (65.2)**	**55 (41.5)**	**617 (45.7)**

n: Number of isolates; **APX:** Ampicillin/cloxacillin; **AZT:** Azithromycin; **CTX:** Ceftriaxone; CHL**:** Chloramphenicol; **CPX:** Ciprofloxacin; **LV:** Levofloxacin; **CN:** Gentamicin: **NB:** Norfloxacin; RD**:** Rifampicin; **STR:** Streptomycin

The degrees of resistance per antimicrobial categories indicate that only one strain (S. *pneumoniae*) exhibited resistance against no antimicrobial category, while 5 strains were resistant to at least one antimicrobial category. The highest number of resistances were found against four antimicrobial categories, while all the strains were found to be resistant to three categories. The 135 bacterial strains recovered from the sputum samples had a cumulative of 85.9% multidrug resistant strains, including 36.3% extensively resistant strains; there were no pan-drug resistance among the strains; while 14.1% were non-multidrug resistant. Three of the isolates namely *Proteus mirabilis,Acinetobacter baumannii* and *Enterobacter cloacae* recorded 100% MDR strains, *Pseudomonas aeruginosa* and *Streptococcus pyogenes* had 91.7% MDR strains, followed by *Klebsiella pneumoniae* 87.5%, *Staphylococcus aureus* 85.75%, *Streptococcus pneumoniae* 80.5% and *Escherichia coli*75.0%. (Table 4)

Table 4: Degrees of Resistance and Multidrug Resistance Patterns in Bacterial obtained from the Sputum of Adults with Community Acquired Pneumonia in Diobu, Port Harcourt, Nigeria

Bacterial Species	**N**	**R 0**	**R 1**	**R 2**	**R 3**	**R 4**	**R 5**	**R 6**	**R 7**	**NMDR %**		**MDR %**		**XDR**	**%**	**PDR**	**%**
P. mirabilis	10	0	0	0	6	3	1	0	0	0	0	10	100	1	10	0	0
E. cloacae	5	0	0	0	2	0	1	2	0	0	0	5	100	3	60	0	0
A. baumanii	2	0	0	0	1	1	0	0	0	0	0	2	100	0	0	0	0
P. aeruginosa	12	0	0	1	2	4	3	2	0	1	8.3	11	91.7	5	41.7	0	0
S. pyogenes	12	0	0	1	2	5	1	3	0	1	8.3	11	91.7	4	33.3	0	0
K. pneumoniae	16	0	0	2	2	6	4	2	0	2	12.5	14	87.5	6	37.5	0	0
S. aureus	21	0	0	3	3	5	4	4	2	3	14.3	18	85.7	10	47.6	0	0
S. *pneumoniae*	41	1	2	5	6	11	10	2	4	8	19.5	33	80.5	16	39.0	0	0
E. coli	16	0	3	1	4	4	3	0	1	4	25.0	12	75.0	4	25.0	0	0
Total	135	1	5	13	28	39	27	15	7	19	14.1	116	85.9	49	36.3	0	0

N: Number of bacterial strains; R0, R1, R2…..R7: Number of resistant strains per antimicrobial category; NMDR: Non-multidrug resistance; MDR: Multidrug resistance; XDR: Extensively drug resistance; PDR: Pan drug resistance

Discussion

This study has successfully contributed to published data on bacteriological profile and antimicrobial resistance patterns in aetiological agents of community acquired pneumonia in Port Harcourt, Nigeria, by identifying nine bacterial species that are associated with CAP and their antimicrobial resistance patterns. The prevalence of bacterial pathogens identified in this study, at 43.8% aligns closely with results obtained elsewhere in Philippines, 40% [18] and Ethiopia, 46.3%[19] but lower that the results obtained in anotherstudy in Ethiopia, 50% [3] Zambia 59% [20] and Iran 64.8% [10]. It is however higher than the 33% [21] and 34.9% [22] in Switzerland and Brazil respectively. The variations in prevalence of bacterial pathogens may be ascribed to various factors such as the status of the healthcare delivery system of the various countries with regards to access to treatment and quality of diagnostic and treatment services, the state of the public health services relating to the prevention and control of diseases, antibiotic use, abuse and misuse, as well as geographical, climatic, socio-demographic, economic and related factors affecting the spread of the diseases and the rate of acquisition and dissemination of bacterial resistomes. They are also likely to be because of differences in sample sizes and products of chance.

The outcomes in this study aligned with those of several previous studies which found *Streptococcus pneumoniae* as predominant isolate from sputum of persons clinically diagnosed with CAP in both immuno-competent [10,23,24] and immuno-compromised patients [19]. Some other bacteria particularly *Klebsiella pneumoniae* have also been reported as most prevalent in other studies. In Ethiopia, *Klebsiella pneumoniae* was reported as most prevalent, followed by *Streptococcus pneumoniae* [7].*Pseudomonas aeruginosa* was reported as the most common isolates in Romanians admitted in hospital with covid 19 associated CAP[25]. The isolates obtained in this study are commonly ubiquitous environmental organisms easily encountered in the community and thus poses great public health risks to immuno-competent and immuno-compromised persons in their daily quest for livelihood.The predominance of *Streptococcus pneumoniae* and *Klebsiella pneumoniae* may be ascribed to their ability to produce capsules which help in circumventing phagocytosis and other activities of the immune

system; in addition to the fact that most of the isolates have been well adapted to existence within the human body as well as the external environment.

The bacterial strains in this study were dominatedby the gram-positive strains with*Streptococcus pneumoniae*and *Staphylococcus aureus*being the commonest isolates; the gram-positive isolates amounted to 54.8% against 45.2% for the gram negatives, which constituted six (66.7%) of the species as against three (33.3%) gam positives. Thisis consistentwith the findings in an Iranian study [10] though different outcomes were reported in some other studies[7,19,26]. The differences may not be of much significance as the same types of bacteria have been repeatedly isolated in several studies. [7,10,19,24,26]

The findings relating to antimicrobial resistance among the isolates reinforced the fact that antimicrobial drug resistance constitutes a disturbing public health challenge in the fight against infectious diseases. Five (55.6%) of the nine isolated species were members of the ESKAPE pathogens, namely *Pseudomonas aeruginosa*,*Staphylococcus aureus*, *Enterobacter cloacae. Klebsiella pneumoniae* and*Acinetobacter baumannii*; it is thus not surprising that the three most resistant species were ESKAPE pathogens. This is a pointer that ESKAPE pathogens remain important public health threats that requires to be more extensively tackled to curtail their widespread negative impacts on the fight against bacterial infections.

The overall MDR prevalence rate of 85.9% observed in this study. aligns closely with the 84.6% reported in a study in Ethiopia [19] but a bit higher than the 63.1% and72.2% reported in Ethiopia [7,27] and far higher than 22% reported in Indonesia [28]. The outcomes indicate clearly that multidrug resistance in bacterial isolates from CAP cases are very high and may be due to the high proclivity of the bacteria to acquire and disseminate resistomes in the external environment where they are free living as well as within the host where they may express pathogenic characters.

Conclusion

The bacteriological profile of the isolates in this study showed a high prevalence rate for bacterial aetiological agents in CAP in adults in Nigeria, while the antimicrobial resistance patterns indicate high and disturbing level of multidrug resistance. This implies that while many people are likely to be infected, most of the antibiotics are losing potency against the bacterial pathogen. This may be attributable to pervasive abuse and misuse of antimicrobial drugs due mainly weak regulatory laws and defective regulation which has spawned reckless abuse and

misuse of drug. Particularly antibiotics in the country. It thus becomes imperative that efforts should be increased in the battle against drug resistance through enhanced education and enlightenment of the populace on the appropriate use of antibiotics and the dangers of abuse and misuse of drugs. There is also the need for regulatory laws on drug usage as it pertains antimicrobial agents to be reviewed and brought up to date while the regulatory agencies should be alive to their duties.

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