Molecular Characterization of Multidrug-resistant Bacteria Isolated From Patients With Pneumonia at Two Hospitals in North-West Nigeria

Paul I Oyegoke1, Busayo O Olayinka1, Joseph O Ehinmidu2, Babajide A Tytler2

1Department of Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria
2Department of Pharmaceutics and Industrial Pharmacy, Ahmadu Bello University, Zaria, Nigeria

Abstract

Background and aims: The spread of antimicrobial resistance (AMR) is a serious public health threat complicating treatment and resulting in prolonged hospitalization. The prevalence of AMR threat is not well defined due to the dearth of appropriate surveillance systems. This study sought to assess the prevalence of AMR among bacterial isolates from sputum specimens obtained from patients with pneumonia presenting at two secondary healthcare facilities in Zaria from June 1 to August 31, 2018.

Methods: Standard methodology was followed in processing sputum samples that met the acceptance criteria. The antibiotic susceptibility patterns of bacterial pathogens cultured from sputum specimens obtained from June 1 to August 31, 2018 were evaluated using the recommendation of the Clinical and Laboratory Standards Institute. Finally, data were analyzed using descriptive statistics.

Results: Acinetobacter spp. were the predominant pathogens accounting for 32% of recovered isolates, followed by Staphylococcus spp. (18%) and Klebsiella spp. (17%), respectively. AMR was found in 91% of the isolates. Most isolates were resistant to erythromycin (ERY) (80%) and amoxicillin (83.3%). Eventually, the multiple antibiotic resistance index ≥0.3 was observed in 76% of the isolates.

Conclusion: Based on the findings, AMR rates were observed to be high, and may display a serious therapeutic challenge to the management of community-acquired pneumonia. Concerted efforts are needed to combat the worrisome AMR trends revealed in this study.

Keywords: Acinetobacter spp., Antibiotic resistance, Community-acquired pneumonia, Klebsiella spp.

Introduction

Of respiratory tract infections, pneumonia remains a major cause of morbidity and mortality in both children and adults, particularly in low and middle-income countries. It kills an estimated 1 million children under the age of 5 every year and accounts for 16% of deaths in preschool children with around 90% occurring in the developing world and low-income countries, where access to medical care is often difficult and the availability of routine vaccination is still below global standards. According to Liu et al, of the 6.3 million reported deaths in children in the first 5 years of life worldwide in 2013, 52% died of infectious disease, and pneumonia has been responsible for about 15% of the total deaths.

In an Australian review of pneumonia in adults aged ≥65 years in 2012, about 78 000 general practitioner visits due to pneumonia were recorded during 2008-2013, and nearly 43 336 pneumonia hospitalizations were along reported in 2011-2012. Pneumonia is a global public health threat and most severe cases have been reported to be of bacteria-associated type. It has been suggested that reduced immune function as a result of comorbidities, previous but resolved viral respiratory infection, and high microbial load during colonization may be predisposing factors to bacterial pneumonia. Streptococcus pneumoniae, Haemophilus influenzae have been reported as predominant bacterial pathogens associated with pneumonia. Gram-negative bacilli (e.g., Klebsiella spp., Acinetobacter spp.) have been demonstrated as the emerging threat. Antibiotic resistance threatens the limited armory of drugs available for treating common infections, a trend which is expected to continue. Empiric antibiotic therapy for pneumonia has consisted of beta-lactams, especially amoxicillin (AML) for non-complicated cases, injectable cephalosporins (e.g., ceftriaxone, CRO) for severe cases, and combinations which could be a macrolide (erythromycin, ERY), aminoglycoside (gentamicin, GEN), or a respiratory fluoroquinolone. However, penicillin-resistant S. pneumoniae, ampicillin-resistant H. influenzae, methicillin-resistant Staphylococcus aureus (MRSA, both community- and hospital-acquired), and multidrug-resistant (MDR) Enterobacteriaceae have been...
implicated in pneumonia cases. Antimicrobial resistance (AMR) increases drug costs and the length of stay and adversely affects the patient’s outcome. Resistance to all classes of antibiotics has developed to various extents among the common and important pathogens. The most frequently reported mechanism of resistance to beta-lactams (common among gram-negative bacteria) is the production of penicillin hydrolyzing enzymes, called beta-lactamas. The presence of beta-lactamas dates back to the pre-antibiotic era, and was first reported just over a decade after the discovery of penicillin.\textsuperscript{19} The first beta-lactamase, TEM, named after a Grecian patient, Temoneira, was first reported in 1963,\textsuperscript{20} and then, the sulfhydryl variable (SHV) beta-lactamase was reported ten years later, which are able to hydrolyze penicillins and narrow-spectrum cephalosporins.\textsuperscript{21}

The extended-spectrum beta-lactamas (ESBLs), which were capable of hydrolyzing penicillin and cephalosporins (especially expanded spectrum cephalosporins), were identified in 1980.\textsuperscript{22} These are believed to have originated from the TEM-1, TEM-2, and SHV-1 enzymes, and differ from their parents by single amino acid substitutions.\textsuperscript{23}

The introduction of beta-lactamase inhibitors to counteract the effect of these enzymes in the 1980s did work out for a while. However, new variants of the enzymes that are not affected by the activity of these beta-lactamase inhibitors abound, and range from the complex mutant of TEM (CMT) which hydrolyze both 3rd generation cephalosporins and possess poor affinity for clavulanic acid, to the metallo-beta-lactamas which are not affected by both avibactam and clavulanic acid.\textsuperscript{24} Currently, there are thousands of identified beta-lactamas, and new discoveries as research into these compounds have a continuous trend.

The other mechanisms of resistance to beta-lactams are the modification of the penicillin-binding proteins (serine acyltransferases that catalyze the formation of cross-linked peptidoglycan and the target of acyltransferases that catalyze the formation of cross-linked peptidoglycan), the production of penicillin modifying enzymes, and the modification of the ribosome of host bacteria, hence, ERY ribosomal methylase (\textit{erm}) genes. These genes coordinate the modification of the ribosome of host bacteria, hence, ERY is unable to bind. They are achieved by the addition of two methyl residues to a highly conserved adenine residue in domain V, the peptidyl transferase center of 235 RNA, leading to a conformational change in the ribosome.\textsuperscript{25}

Several variants of these genes have so far been identified in this regard. The \textit{ermA} gene, commonly found among Staphylococci spp., the \textit{ermB} gene is reported in both Gram-negative and -positive organisms while the \textit{ermC} gene is detected among organisms.\textsuperscript{26}

Another mechanism of macrolide resistance is the efflux pump mediated by the \textit{msr} and \textit{mef} genes, which code for low-level resistance,\textsuperscript{27} however, high-level macrolide resistance is expressed in combination with \textit{erm} genes. Macrolide-inactivating phosphotransferases are coded for by \textit{mph}, which are arranged in tandem and expressed from the same promoter as the macrolide efflux pump.\textsuperscript{30}

Therefore, the aim of this study is to determine the frequency of microbial agents and their antibiotic resistance patterns in pneumonia over a 3-month period (June 1 to August 31, 2018) in Zaria, Nigeria.

Materials and Methods

This study was performed by focusing on two hospitals in Zaria metropolis viz Hajiya Gambo Sawaba hospital, and Kofan-gaya, Zaria, Kaduna State, Nigeria. This is a 200-bed capacity facility with over 5000 admissions annually and is located within Zaria, the Zaria Local Government area while Saint Luke’s Anglican Hospital is located in Wusasa, Kaduna State, Nigeria, and is a private secondary health care provider with over 3000 admissions annually. Patients of all age groups presenting at the hospital and diagnosed as having pneumonia by a physician. Ninety patients with community-acquired pneumonia presenting at the two care facilities were included in the study.

Sample Collection, Processing and Identification of Organisms

The collected sputum specimens from June 1 to August 31, 2018 were processed using standard methods. Blood, MacConkey, and Mannitol salt agars (Titan Biotech Ltd, India) were used for the isolation of bacteria. The samples were inoculated onto prepared agar plates, which were incubated at 37°C for 24 hours in an aerobic atmosphere. Standard biochemical tests using Microgen ID kits (Microgen Bioproducts Ltd, UK) were employed to identify the organisms.

Antibiotic Susceptibility Testing

The bacteria were tested against a panel of antibiotics using the guidelines of the Clinical Laboratory Standards Institute,\textsuperscript{31} and the tested antibiotics were purchased from Oxoid Ltd. (Basingstoke, Hampshire, England) and included GEN (30 \textmu g), streptomycin (S, 30 \textmu g), and amoxicillin-clavulanate (AMC, 30 \textmu g). Other antibiotics were AML (30 \textmu g), vancomycin (VAN, 30 \textmu g), oxacillin (OXA, 10 \textmu g), CRO (30 \textmu g), ceftazidime (CAZ, 30 \textmu g), cefoxitin (FOX, 30 \textmu g), ciprofloxacin (CIP, 5 \textmu g), and trimethoprim-sulfamethoxazole (SXT, 1.25 \textmu g + 23.75 \textmu g). In addition, ERY (15 \textmu g), azithromycin (AZT, 15 \textmu g), linezolid (LZD, 10 \textmu g), quinupristin-dalfopristin (QD, 15 \textmu g), tetracycline (TET, 30 \textmu g), and imipenem (IMP, 10 \textmu g) were other employed antibiotics. The modified Kirby-Bauer disc-diffusion method was used to determine the antibiotic susceptibility of isolates identified and confirmed by biochemical tests. An overnight culture of each isolate was prepared in nutrient agar and incubated at 37°C for 18 hours. Five milliliters (5 mL)
of sterile physiological saline and 0.5 McFarland turbidity standard solutions were prepared for the standardization of inoculums. The discrete colonies of isolates on nutrient agar plates were emulsified in 5 mL of sterile physiological saline and the turbidity adjusted to 0.5 McFarland standard (approximately a cell density of $1.5 \times 10^8$ cfu/mL). The standardized suspension was inoculated on Muller-Hinton agar at an angle 60º across the plate using a sterile cotton swab to ensure even distribution and confluent growth. The plates were allowed to dry for 5 minutes. The disc of various antibiotics was aseptically placed using a sterile forceps on the dried inoculated agar surface. After 30 minutes of applying the discs to allow for pre-diffusion, the plates were incubated at 37°C for 18 hours. After incubation, the plates were examined for the zones of inhibition and result interpretation according to the Clinical and Laboratory Standards Institute (CLSI). The multiple antibiotic resistance index (MARI), which is an indicator of the level of exposure to antibiotics, was determined as well. \[ MAR\text{ Index} = \frac{\text{Number of antibiotics to which resistant}}{\text{Total number of antibiotics tested}} \]

For the purpose of this study, non-susceptibility to one or more antibiotic(s) in at least three classes was considered as multidrug resistance.

Statistical Analysis
IBM Statistical Package for Social Sciences (Version 22, International Business Machines Corporation) was used for data analysis, and the results are presented as frequencies, percentages, and means.

Results
The gender distribution of diagnosed pneumonia cases is provided in Table 1. A total of 49 males (54.4%) and 41 females (45.6%) were sampled within the period, giving a male-female ratio of 1.2. The highest and the lowest number of samples were collected from 16-49 and 6-15 age groups, respectively. No samples were collected from children aged <5.

Of the ninety collected sputum specimens, seventy-eight were positive for bacterial growth. Gram-negative bacteria made up more than 70% of the recovered isolates (Table 2). *Acinetobacter* spp. (*A. baumannii* (06), *A. Iwoffii* (16) and *A. haemolyticus* (03)), *Staphylococcus* spp., and *Klebsiella* spp. (*K. pneumoniae* (09), *K. ornithinolytica* (02), *K. oxytoca* (01), and *K. ozaenae* (02)) were the predominant isolates.

Based on the results (Table 3), most Gram-negative isolates exhibited resistance to AML and ERY. *Acinetobacter* spp. showed high resistance to ERY (65%), AMC (45%), and TMP/SMX (35%).

*Klebsiella* spp. were resistant to ERY (100%), AML (92.3%), CRO (77%), FOX (30%), TMP/SMX (30%), and TET (23%). One *Klebsiella* isolate demonstrated resistance to IMP. *Serratia* spp. were resistant to ERY (100%), AML (100%), ceftazidime (100%), FOX (100%), TET (60%), and TMP/SMX (60%). *Enterobacter gergoviae* represented high resistance to ERY (100%), AML (100%), CRO (50%), FOX (67%), TET (50%), and TMP/SMX (50%). The results also revealed that *Hafnia alvei* was resistant to the tested antibiotics including IMP, CIP, IMP, and GEN each showed broad activity against most gram-negative isolates.

Of the gram-positive isolates (i.e., *Streptococcus* spp.) demonstrated no resistance to any of tested antibiotics. Coagulase positive *Staphylococcus* spp. indicated high resistance to ERY (78%), LZD (78%), quinupristin-dalfopristin (QD, 78%), AML (78%), oxacillin (56%), TET (44%), TMP/SMX (44%), S (33%), and CIP (22%). Coagulase negative *Staphylococcus* spp. showed high resistance to ERY (100%), QD (100%), AML (100%), oxacillin (100%), TET (100%), TMP/SMX (100%), S (100%), and CIP (100%). Further, 50% of the *Staphylococcus* spp. isolates were methicillin resistant.

Based on the findings, 91% of the isolates were resistant to at least one antibiotic with multidrug resistance observed in 47% of the isolates while only 9.1% of them were susceptible to all tested antibiotics. The percentage of multidrug resistance shown by the isolates is presented in Table 4.

Table 5 provides the MARI of bacterial isolates. In this study, 75.8% of organisms indicated an MARI of greater than or equal to 0.3. Ten MDR isolates were investigated
for the carriage of resistance genes. The molecular detection of the *ESBL* and *ermB* genes revealed that 7 isolates were positive for the *TEM* gene, with 5 harbouring the *ermB* gene while 3 of them were positive for *OXA* (Figure 1 and Table 6, respectively).

### Discussion

Pneumonia ranks high among the killer infectious diseases of children and adults, affecting both low- and high-resource countries. Mortality rates are kept at the minimum by a combination of early diagnosis, appropriate treatment protocols, and the diligent management of associated comorbidities and risk factors.34,35

In this study, Gram-negative bacilli were the predominant isolates, which is in line with reports in south-western Nigeria36,37 and Cambodia.38 Microbial communities are known to vary with the geographic location. In this study, the majority of the recovered ones were Gram-negative bacilli, which corroborates with reports in Indonesia39 and Bangladesh.40 Gram-positive cocci, however, was reported as being predominant in Bosnia41 and Sweden.42

Bacteria-associated pneumonia, which accounts for the most severe cases, is complicated by the threat of antibiotic resistance, which is also found in our study. The observed resistance levels to AML (45-100%) and ERY (65-100%) were worrisome, considering that beta-lactams, particularly AML, are generally adopted as first-line drugs in empirical treatment, occasionally in combination with macrolides (e.g., ERY).43

The prevalence of MDR isolates (47%) was high in this study, which could be due to over-exposure to and the possible misuse of antibiotics. El-Sokkary et al44 reported higher MDR rates in a similar study in Egypt. *Staphylococcus* spp., *Acinetobacter* spp., and *Klebsiella* spp. accounted for most of the MDR isolates in this study, which is consistent with the reports of extensively drug-resistant *Acinetobacter*.
spp. isolated from pneumonia patients.\textsuperscript{45} Observed methicillin resistance in \textit{Staphylococcus} spp. was higher compared to reports of 33.3\% from Ethiopia.\textsuperscript{46} MRS spp. have previously been implicated in nosocomial cases although a recent report revealed community-acquired – MRS pneumonia, particularly CA-MRSA.\textsuperscript{47} The low resistance of \textit{Staphylococcus} species to GEN and CIP in this study indicated that these could be a useful alternative for treatment in cases where first-line antibiotics represent a failure. An Ethiopian study reported similar findings.\textsuperscript{47} There was no resistance to any of the tested antibiotics in \textit{Streptococcus} spp., which is contrary to the results of studies from Egypt and Ethiopia\textsuperscript{44,47} regarding resistance.

Most Gram-negative isolates were resistant to ERY and AML although GEN, CIP, and IMP could be alternatives for MDR bacteria-associated pneumonia cases within these settings since there was less resistance. El-Sokkary et al\textsuperscript{44} reported similar effectiveness of carbapenems and fluoroquinolones in Egypt although it contrasts with the significant resistance to amikacin and CIP reported in India.\textsuperscript{44}

The presence of carbapenem-resistant Enterobacteriaceae in this study is of public health importance because the treatment of infections caused by these organisms is extremely difficult.\textsuperscript{49} Carbapenem-resistant Enterobacteriaceae in pneumonia patients has been reported as well.\textsuperscript{50} Resistance to IMP is low in these setting, as observed in this study, probably due to controlled prescription and high costs of these antibiotics making them inaccessible to over-the-counter self-medication, the possibility of transfer of carbapenem-resistant genes to susceptible bacteria remains, and could be deleterious.

The high level of antibiotic exposure in the community could be responsible for the high observed MARI. A similar figure was reported in children with suspected septicemia presenting at the Institute of Child Health, Zaria.\textsuperscript{51} The observed resistance rates in this study are worrisome, and the possibility of the transfer of resistance determinants to drug-susceptible bacteria presents a looming danger. Policies for reducing the development and spread of AMR in bacteria pathogens include antimicrobial stewardship, discouraging misuse, overuse and indiscriminate over-the-counter antibiotic prescriptions, and encouraging patients’ medication compliance.

The code of molecular resistance determinants for specific antibiotic non-susceptibilities in some cases contributes to cross-resistance between antibiotics within the same or different class thus limiting treatment options.\textsuperscript{52} The detection of \textit{TEM-1} and \textit{OXA-1} genes in seven (7) and three (3) of the tested (10) isolates suggests possible inclinations with encountered AML and CRO resistance in the selected isolates, respectively. The detection of OXA and TEM genes in two (2) of the isolates suggests the presence of multiple resistance mechanisms. \textit{TEM} and \textit{OXA} genes are known to mediate beta-lactam resistance through the production of enzymes that inactivate the antibiotic.\textsuperscript{53,54} Beta-lactams are generally classified into narrow and broad spectrums although these gene-mediated enzymes have evolved producing variants which act on them making them ineffective.\textsuperscript{25} Infections caused by organisms possessing these genes can increase the length of hospital stay and result in intensive care unit admission. Early detection is important because the inappropriate therapy of these complex infections can increase mortality and morbidity.

The presence of \textit{ermB} genes conferring resistance to macrolides is of importance to physicians’ prescription patterns.\textsuperscript{35} Treatment protocols may need to be reassessed to forestall community or hospital epidemic of treatment failures due to antibiotic resistance. To the best of our knowledge, there were no available reports on the molecular characterization of \textit{ESBLs} and ERY ribosomal methylase (\textit{ermB}) genes from the sputum of patients with

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Isolates} & \textbf{TEM (1150 bp)} & \textbf{OXA (813 bp)} & \textbf{ermB (639 bp)} \\
\hline
H01 & + & - & + \\
H06 & + & - & - \\
H12 & + & - & + \\
S04 & + & - & - \\
S06 & - & - & + \\
S21 & - & - & - \\
S22 & - & + & - \\
S36 & + & + & + \\
S42 & + & - & + \\
S46 & + & + & - \\
\hline
\end{tabular}
\caption{Detection of TEM, OXA, and \textit{ermB} Genes in MDR Bacterial Isolates}
\end{table}
pneumonia in Nigeria at the time of compilation of this report. However, a similar study in Japan reported the isolation of ESBL genes from the sputum of pneumonia patients.\textsuperscript{56} Another report of the isolation of OXA type and TEM genes in the Acinetobacter baumannii isolate from the sputum of the neonatal pneumonia patient was found in China.\textsuperscript{57} The presence of TEM, OXA, and \textit{ermB} genes pose a significant threat to the current antibiotic therapy for pneumonia since this could culminate in treatment failures leading to a prolonged hospital stay and fatal outcomes.

**Conclusion**

The results of this study showed that AMR rates were observed to be high and may display a serious therapeutic challenge to the management of community-acquired pneumonia. Thus, concerted efforts are needed to combat the worrisome AMR trends revealed in this study.

**Conflict of Interest Disclosures**

The authors declared no conflict of interests.

**Ethical Approval**

Ethical approval was obtained from the Ethics Committee of the Health and Research of the Kaduna State Ministry of Health (MOH/ADM/744/VOL.1/462).

**References**

1. UNICEF. Nigeria Contributes Highest Number to Global Pneumonia Child Deaths. UNICEF; 2019. https://www.unicef.org/nigeria/press-releases/nigeria-contributes-highest-number-global-pneumonia-child-deaths. Accessed December 12, 2019.

2. WHO Pneumonia fact sheet. World Health Organisation 2016. http://www.who.int/mediacentre/factsheets/fs331/en. Accessed May 1, 2018.

3. Rudan I, O’Brien KL, Nair H, Liu L, Theodoratou E, Qazi S, et al. Epidemiology and etiology of childhood pneumonia in 2010: estimates of incidence, severe morbidity, mortality, underlying risk factors and causative pathogens for 192 countries. J Glob Health. 2013;3(1):010401. doi: 10.7189/jogh.03.010401.

4. Zar HJ, Madhi SA, Aston SJ, Gordon SB. Pneumonia in low and middle income countries: progress and challenges. Thorax. 2013;68(11):1052-6. doi: 10.1136/thoraxjnl-2013-204247.

5. Izadnegahdar R, Cohen AL, Klugman KP, Qazi SA. Childhood pneumonia in developing countries. Lancet Respir Med. 2013;1(7):574-84. doi: 10.1016/s2213-2600(13)70075-4.

6. Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, et al. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet. 2015;385(9966):430-40. doi: 10.1016/s0140-6736(14)61698-6.

7. Earle K, Williams S. Burden of pneumococcal disease in adults aged 65 years and older: an Australian perspective. Pneumonia (Nathan). 2016;8:9. doi: 10.1186/s41479-016-0008-8.

8. Bhuiyan MU, Snelling TL, West R, Lang J, Rahman T, Borland ML, et al. Role of viral and bacterial pathogens in causing pneumonia among Western Australian children: a case-control study protocol. BMJ Open. 2018;8(3):e020646. doi: 10.1136/bmjopen-2017-020646.

9. Mizgerd JP. Respiratory infection and the impact of pulmonary immunity on lung health and disease. Am J Respir Crit Care Med. 2012;186(9):824-9. doi: 10.1164/rcrm.201206-1063PP.

10. Metzger DW, Sun K. Immune dysfunction and bacterial coinfections following influenza. J Immunol. 2013;191(5):2047-52. doi: 10.4049/jimmunol.1301152.

11. Lee KH, Gordon A, Foxman B. The role of respiratory viruses in the etiology of bacterial pneumonia: an ecological perspective. Evol Med Public Health. 2016;2016(1):95-109. doi: 10.1093/emph/emw007.

12. Griffiths C, Drews SJ, Marchant DJ. Respiratory syncytial virus: infection, detection, and new options for prevention and treatment. Clin Microbiol Rev. 2017;30(1):277-319. doi: 10.1128/cmwr.00010-16.

13. Goyet S, Vlieghe E, Kumar V, Newell S, Moore CE, Bousfield R, et al. Etiologies and resistance profiles of bacterial community-acquired pneumonia in Cambodian and neighboring countries' health care settings: a systematic review (1995 to 2012). PLoS One. 2014;9(3):e89637. doi: 10.1371/journal.pone.0089637.

14. Narula S, Sharma P, Kumar N, Kumar N, Kumar M. An upsurge of gram negative bacteria in community acquired pneumonia: an alarming trend! J Emerg Med Forecast. 2018;1:1007.

15. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health. 2015;109(7):309-18. doi: 10.1179/2047773215y.0000000030.

16. Queen MA, Myers AL, Hall M, Shah SS, Williams DJ, Auger KA, et al. Comparative effectiveness of empiric antibiotics for community-acquired pneumonia. Pediatrics. 2014;133(1):e23-9. doi: 10.1542/peds.2013-1773.

17. Lee MS, Oh JW, Kang CJ, Kim ES, Park S, Rhee CK, et al. Guideline for antibiotic use in adults with community-acquired pneumonia. Infect Chemother. 2018;50(2):160-98. doi: 10.3947/ic.2018.50.2.160.

18. Self WH, Wunderink RG, Williams DJ, Zhu Y, Anderson EJ, Balk RA, et al. \textit{Staphylococcus aureus} community-acquired pneumonia: prevalence, clinical characteristics, and outcomes. Clin Infect Dis. 2016;63(3):300-9. doi: 10.1093/cid/ciw300.

19. Abraham EP, Chain E. An enzyme from bacteria able to destroy penicillin. Nature. 1940;146(3713):837-7. doi: 10.1038/146837a0.

20. Datta N, Richmond MH. The purification and properties of a penicillinase whose synthesis is mediated by an R-factor in penicillin-resistant \textit{Pseudomonas aeruginosa}. J Gen Microbiol. 1972. p. 15-93. doi: 10.1007/3-540-05814-1_2.

21. Abraham EP, Chain E. An enzyme from bacteria able to destroy penicillin. Nature. 1940;146(3713):837-7. doi: 10.1038/146837a0.

22. Knothe H, Shah P, Krcmery V, Antal M, Mitsuhashi S. Mechanisms of bacterial resistance to antibiotics. In: Reviews of Physiology. Vol 65. Berlin, Heidelberg: Springer; 1972. p. 15-93. doi: 10.1007/3-540-05814-1_2.

23. Bush K. Past and present perspectives on \textbeta-lactamases.
Antimicrob Agents Chemother. 2018;62(10):e01076-18. doi: 10.1128/aac.01076-18.

24. Bush K, Bradford PA. β-Lactams and β-lactamase inhibitors: an overview. In: Silver LL, Bush K, eds. Antibiotics and Antibiotic Resistance. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 2016. p. 23-44.

25. Palzkill T. Structural and mechanistic basis for extended-spectrum drug-resistance mutations in altering the specificity of TEM, CTX-M, and KPC β-lactamases. Front Mol Biosci. 2018;5:16. doi: 10.3389/fmolb.2018.00016.

26. Sun S, Selmer M, Andersson DI. Resistance to β-lactam antibiotics conferred by point mutations in penicillin-binding proteins PBPs3, PBPs4 and PBPs6 in Salmonella enterica. PLoS One. 2014;9(5):e97202. doi: 10.1371/journal.pone.0097202.

27. Wilson DN. Ribosome-targeting antibiotics and mechanisms of bacterial resistance. Nat Rev Microbiol. 2014;12(1):35-48. doi: 10.1038/nrmicro3155.

28. Fylle C, Grossman TH, Kerstein K, Sutcliffe J. Resistance to macrolide antibiotics in public health pathogens. Cold Spring Harb Perspect Med. 2016;6(10):a025395. doi: 10.1101/cshperspect.a025395.

29. Zhang Y, Tsutomo O, Okada R, Hata N, Matsumoto M, Isaka M, et al. Predominant role of msr(D) over mef(A) in macrolide resistance in Streptococcus pyogenes. Microbiology (Reading). 2016;162(1):46-52. doi: 10.1099/mic.0.00206.0.

30. Morar M, Pengelly K, Koteva K, Wright GD. Mechanism and diversity of the erythromycin esterase family of enzymes. Biochemistry. 2012;51(8):1740-51. doi: 10.1021/bi201790u.

31. Clinical & Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI Document M100-S28. Wayne, PA: CLSI; 2018.

32. Krumpsperman PH. Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of fecal contamination of foods. Appl Environ Microbiol. 1983;46(1):165-70. doi: 10.1128/aem.46.1.165-170.1983.

33. aul S, Bezbahar RL, Roy MK, Ghosh AC. Multiple antibiotic resistance (MAR) index and its reversion in Pseudomonas aeruginosa. Lett Appl Microbiol. 1997;24(3):169-71. doi: 10.1046/j.1472-765x.1997.00364.x.

34. Messinger AI, Kupfer O, Hurst A, Parker S. Management of Pseudomonas aeruginosa β-lactamase inhibitors: an overview. J Antimicrob Chemother. 2018;73(9):2433-44. doi: 10.1093/jac/dky257.

35. Agwewu A, Lilford RJ, English M. Appropriateness of clinical severity classification of new WHO childhood pneumonia guidance: a multi-hospital, retrospective, cohort study. Lancet Glob Health. 2018;6(1):e74-e83. doi: 10.1016/s2214-109x(17)30448-5.

36. Okesola AO, Ige OM. Trends in bacterial pathogens of lower respiratory tract infections. Indian J Chest Dis Allied Sci. 2008;50(3):269-72.

37. Akingbade OA, Ogiogwa JA, Okentugbua PO, Innocent-Adiele HC, Onoh CC, Nwanze JC, et al. Prevalence and antibiotic susceptibility pattern of bacterial agents involved in lower respiratory tract infections in Abeokuta, Ogun State, Nigeria. Rep Opinion. 2012;4(5):25-30.

38. Inghammar M, By Y, Farris C, Phe T, Borand L, Kerleguer A, et al. Serotype distribution of clinical Streptococcus pneumoniae isolates before the introduction of the 13-valent pneumococcal conjugate vaccine in Cambodia. Am J Trop Med Hyg. 2018;98(3):791-6. doi: 10.4269/ajtmh.17-0692.
Zaria, Nigeria. Bayero J Pure Appl Sci. 2016;9(2):114-20. doi: 10.4314/bajopas.v9i2.22.

52. Sultan I, Rahman S, Jan AT, Siddiqui MT, Mondal AH, Haq QMR. Antibiotics, resistome and resistance mechanisms: a bacterial perspective. Front Microbiol. 2018;9:2066. doi: 10.3389/fmicb.2018.02066.

53. Evans BA, Amyes SG. OXA-β-lactamases. Clin Microbiol Rev. 2014;27(2):241-63. doi: 10.1128/cmr.00117-13.

54. Mehrad B, Clark NM, Zhanel GG, Lynch JP 3rd. Antimicrobial resistance in hospital-acquired gram-negative bacterial infections. Chest. 2015;147(5):1413-21. doi: 10.1378/chest.14-2171.

55. Haran JP, Volturo GA. Macrolide resistance in cases of community-acquired bacterial pneumonia in the emergency department. J Emerg Med. 2018;55(3):347-53. doi: 10.1016/j.jemermed.2018.04.031.

56. Horie H, Ito I, Konishi S, Yamamoto Y, Yamamoto Y, Uchida T, et al. Isolation of ESBL-producing bacteria from sputum in community-acquired pneumonia or healthcare-associated pneumonia does not indicate the need for antibiotics with activity against this class. Intern Med. 2018;57(4):487-95. doi: 10.2169/internalmedicine.8867-17.

57. Lv W, Zhang X, Hou M, Han D, Li Y, Xiong W. Draft genome sequence of an OXA-23, OXA-66, ADC-25 and TEM-1D co-producing Acinetobacter baumannii ST195 isolated from a patient with neonatal pneumonia in China. J Glob Antimicrob Resist. 2019;16:1-3. doi: 10.1016/j.jgar.2018.11.008.