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Profile and Antimicrobial Resistance Patterns of Bacteria isolated from Patients with Respiratory Tract Infections in Ile-Ife Metropolis

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JO, OO, BO, GN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JO, OO and BO managed the analyses of the study. Authors JO, BO and OO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Respiratory tract infections (RTIs) are public health concerns in developing countries. These infections are usually treated with antibiotics, and in most cases, treatment is initiated before the final laboratory reports are available. Lately, empiric treatment has been complicated by the emergence of resistance and a definitive diagnosis and susceptibility testing would be required for effective management. This study focused on the causative agents of RTIs in Ile-Ife, and the susceptibility patterns of all the isolates.

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Methods: Two hundred and thirteen samples of sputum, pleural aspirate and throat swab were collected aseptically from patients and cultured on the appropriate media. Bacterial isolates were identified by biochemical tests and antimicrobial susceptibility testing of the isolates was determined by Kirby-Bauer disc diffusion technique.
Results: In this study, the prevalence of respiratory tract infections was 38.5%. Male patients and those in the age group of 21-30 years were more vulnerable to respiratory tract infections ($p<0.05$). The highest number of pathogens (n=70; 84.4%) was obtained from sputum, while 12 (24.6%) were isolated from pleural aspirate and throat swab. The commonest bacterial sp isolated was *Klebsiella* sp (n=32; 39%), followed by *Pseudomonas aeruginosa* (n=16; 19.6%), *Mycobacterium tuberculosis* (n=11; 13.4%), *Streptococcus pneumoniae* (n=7; 8.5%), and *Staphylococcus aureus* (n=6; 7.3%). Most of the isolates (>60%) were resistant to amoxicillin, cotrimoxazole, cloxacillin and tetracycline, but far fewer to ofloxacin (<10%).
Conclusion: The level of antibiotic resistance observed in this study is a serious public health problem and hence, underscores the need for timely and proper diagnosis of respiratory tract infections and susceptibility testing.

Keywords: Respiratory tract infections; antimicrobial resistance; bacteriological profile.

1. INTRODUCTION

Respiratory tract infections (RTIs) are considered as a major public health concern and are usually ranked second to diarrhea in morbidity and mortality in many developing countries [1,2,3]. They account for more than 50 million deaths each year in both community and health care settings [4]. RTIs can arise from other people by cross-infection or from environmental sources.

The RTIs can be divided into upper respiratory tract infections that affect the upper respiratory tract and lower respiratory tract infections that affect the lower passageways [5]. The most common infections are limited to the upper respiratory tract and only 5% involve the lower respiratory tract. Upper respiratory infections (URTIs) involve the nose, the paranasal sinuses, pharynx and larynx [5,6,7]. The nasal discharge associated with cold contains virus particles, dead cells from the nasal mucosa and bacteria. In general, most of the upper respiratory tract infections are caused by viruses [8]. The localized infections of oropharynx, nasopharynx, and nasal cavity cause sore throat, nasal discharge and often fever, but throat pathogen may spread to the larynx, causing hoarseness, the middle ear, causing otitis media with earache, paranasal sinus, causing sinusitis with pain in the face or head, eye causing conjunctivitis or keratitis [9]. Lower respiratory tract infections (LRTIs) involve the bronchi and alveoli [10]. They include two serious conditions: acute bronchitis and pneumonia. Inflammation of the lung is a serious condition, responsible for most of the deaths caused by infection of the respiratory tract, especially in adults and infants. Lower RTIs can be caused by pathogens that are inhaled from the environment, spread from blood, or from the upper respiratory tract [5]. Unlike most of the upper respiratory tract, the trachea, bronchi, and lungs are normally free from colonization with commensal and potentially pathogenic bacteria, but when their defenses are compromised, they are liable to be invaded by microorganism from the throat [9].

The common bacteria species that are implicated in RTIs are *Pseudomonas* spp., *Streptococcus* spp., *Proteus* spp., *Klebsiella* spp., *Staphylococcus* spp., *Enterobacter* spp., *Acinetobacter* spp., and *Haemophilus influenzae* [11,12,13]. Other less common cause of bacterial RTIs includes group C beta hemolytic Streptococci, *Corynebacterium diphtheriae*, and *Neisseria gonorrhoeae*.

RTIs are usually treated with antibiotics, and in most cases, there is a need to start antibiotic treatment before the final laboratory reports are available. Lately, empiric treatment has been complicated by the appearance of antimicrobial resistance among the principal pathogens and a definitive bacteriological diagnosis and susceptibility testing would, therefore, be required for effective management [4,14]. In developing countries, most respiratory tract infections are treated empirically, possibly because of higher cost of laboratory services where available.

The emergence of antimicrobial resistance in the management of respiratory tract infections is a serious public health issue, particularly in the developing world. Apart from high levels of poverty, ignorance and poor hygiene practices, there is also high prevalence of fake drugs in circulation. Antibiotic resistance often leads to therapeutic failure of empirical therapy, which is why knowledge of etiologic agents of RTIs and their sensitivities to available drugs are of immense importance to the selection and use of antimicrobial agents [15]. The study was carried out in order to isolate and identify common bacterial spp that are responsible for respiratory tract infections in Ile-Ife metropolis, and to determine the susceptibility patterns of all the isolates to commonly used antimicrobial agents.

2. MATERIALS AND METHODS

2.1 Study Population

Two hundred and thirteen subjects with symptoms of respiratory tract infections within the age group of one and above seventy one (71+) participated in the study from January to December, 2005. Informed consent was obtained from each patient prior to sample collection.

2.2 Collection of Samples and Processing

The patients' sputum samples and pleural aspirates were collected with sterile universal bottles. The patients were instructed not to eat before collection of early morning sputum; saliva was rejected. Throat swabs were also collected aseptically with sterile swab sticks. All samples were sent to the laboratory for processing. Macroscopic appearance of the sputum samples were noted and reported as purulent, mucopurulent, mucoid, muco saliva, and blood stained. The sputum samples were homogenized with sterile Ringer's solution in a sterile universal bottle containing sterile glass beads and incubated at room temperature for 1 hour. The homogenized sputum samples and throat swabs were cultured directly on 0.02% bacitracin chocolate, blood and MacConkey agar plates. Chocolate agar plates were incubated aerobically in a candle jar at 35-37ºC for 18-24 hours. Pleural fluids and aspirates were processed by first noting the appearance and quality of the fluids and then cultured on chocolate, MacConkey and blood agar. Smears were also prepared for Gram and Ziel Neelsen staining techniques.

2.3 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of the isolates was performed by the Kirby-Bauer disc diffusion technique on Mueller Hinton agar (CM0337) (Oxoid Ltd., Basingstoke, Hampshire, England). Antibiotics tested included Amoxicillin (25 µg), Ceftriaxone (30 µg), Chloramphenicol (10µg), Co-trimoxazole (25 µg), Erythromycin (10 µg), Genta-micin (10 µg), Ciprofloxacin (5 µg), Ofloxacin (30 µg), Perfloxacin (30 µg), Tetracycline (30 µg), Amoxicillin/clavulanic acid (30 µg), and Cloxacillin (10 µg) (Remel, U.S.A). The inoculated plates were incubated at 37ºC for 24 hours. Interpretation of the diameters of the zones of inhibition was done according to the guidelines set by clinical and laboratory standard institute (CLSI) [16].

2.4 Optochin Susceptibility Test

The test was performed to differentiate *Streptococcus pneumoniae* from viridans Streptococci. Isolate to be tested were incubated overnight on blood agar plates with optochin discs (Rosco Diagnostica, Denmark) in CO_2 and O_2 atmospheres. Optochin sensitivity and resistance were defined as zones of inhibition of ≥18 mm and <16 mm (upon CO_2 incubation) or ≥20 mm and <18 mm (upon O_2 incubation), respectively.

2.5 Statistical Analysis

The Chi square ($\chi 2$) and Fischer's exact test (two tailed), performed using SPSS statistical software (version 22) (Chicago, IL, USA) were used to determine the statistical significance of the data. All reported p-values were two-sided and a p-value of less than 0.05 was considered to be statistically significant.

3. RESULTS

Of two hundred and thirteen (213) samples that were screened for bacterial spp**.,** 82 (38.5%) yielded pathogens, 100 (46.9%) yielded normal flora while 31 (14.5%) had no growth. Of 82 (38.5%) pathogens detected in the samples, 70 (85.4%) were from sputum and six (7.3%) were from throat swab and pleural aspirate. Of 100 samples that yielded normal floral, 93 (93%) and 7 (7%) were isolated from sputum, and throat swab respectively. No isolate was recovered from nine (29%) sputum samples, 20 (64.5%) pleural aspirates and two (6.5%) throat swabs (Table 1).

As shown in Table 2, the commonest bacterial sp isolated was *Klebsiella* sp. (n=32; 39%), followed by *Pseudomonas aeruginosa* (n=16; 19.6%), *Mycobacterium tuberculosis* (n=11; 13.4%), *Streptococcus pnuemoniae* (n=7; 8.5%), and *Staphylococcus aureus* (n=6; 7.3%). No isolate of *Pseudomonas aeruginosa, Mycobacterium tuberculosis, Proteus* sp *and Escherichia coli* was detected in throat swabs and pleural aspirates, while *Klebsiella* isolates were detected in all clinical specimens. Subjects in the age group 21-30 years old (n=17; 20.7%) were most vulnerable to RTIs followed by those in the age groups 31-40 years old (n=15; 18.2%), 51-60 years old (n=14; 17.0%) and 41-50 years old (n=13; 15.9%). The percentage of bacterial isolates was significantly higher in the age group 21-30 years old compared with age group 31-40 years old (P<0.05) (Table 3).

Although, no statistical significant difference was observed between the frequencies of individual isolates in relation to sex, higher rates of isolation of *Klebsiella* sp., *Pseudomonas aeruginosa, Mycobacterium tuberculosis, Streptococcus pneumoniae* were detected in male patients compared with female patients (p>0.05). In general, more bacterial isolates were recovered from male patients compared with female patients (χ^2=9.756; p= 0.002) (Table 4).

The antimicrobial resistance profile of pathogens from the subjects is shown in Table 5. The isolates (>60%) were commonly resistant to amoxicillin, cotrimoxazole and tetracycline. The least resistance was exhibited to ofloxacin (<10%). None of the isolates of *Proteus* sp, *Streptococcus pneumoniae* and *Staphylococcus aureus* was resistant to ofloxacin. Also, none of the isolates of *Klebsiella* was resistant to perfloxacin. *Pseudomonas aeruginosa* was commonly resistant to amoxicillin/clavulanic acid (n=13; 81.3%) and ceftriaxone (n=12; 75%) while *Streptococcus pneumoniae* were resistant to gentamicin (n=7; 100%), cloxacillin (n=5; 71.4%) and chloramphenicol (n=6; 85.7%).

4. DISCUSSION

Respiratory tract infections are a major health care concern in both developed and developing countries and they are usually ranked second only to diarrhea in mortality and morbidity. [17,1,2,3] The study focused on the causative agents of respiratory tract infections in Ile-Ife metropolis, and their susceptibility patterns.

In this study, the prevalence of respiratory tract infections in Ile-Ife was 38.5%. This is comparable to figures between 20% and 59% in previous reports.[18,19] The occurrence of respiratory tract infections was significantly higher in male patients (62.2%) than in female patients (38.8%) (p<0.05). This finding may be attributed to the involvement of males in various activities such as smoking and consumption of alcohol, which may decrease local immunity in the respiratory tract [20]. Subjects that are within the age group of 21-30 years old had the highest respiratory tract infections (20.7%) followed by the age group of 31-40 years old (18.2%) and 51-60 years old (17.0%) which is in agreement with a previous study by Taura et al.[21] that reported the highest occurrence in the age group 20-29 years old.

Table1. Frequency of isolation of bacteria in clinical samples

Sample	Pathogen n (%)	Normal Flora n (%)	No growth n (%)	Total n (%)
Sputum	70(85.4)	93 (93)	9 (29)	172 (80.8)
Throat swab	6 (7.3)	7 (7)	2 (6.5)	15 (7)
Pleural aspirate	6 (7.3)	0(0)	20 (64.5)	26 (12.2)
Total	82 (38.5)	100(46.9)	31 (14.5)	213 (100)

Table2. Prevalence of bacterial isolates in clinical samples

Bacterial isolates	Sputum	Throat swab	Pleural aspirate	Total n (%)
Klebsiella sp	24	4	4	32 (39.9)
Pseudomonas aeruginosa	16	0	0	16 (19.6)
Mycobacterium tuberculosis	11	0	0	11 (13.4)
Proteus sp	5	0	0	5 (6.1)
Escherichia coli	5	0	0	5 (6.1)
Streptococcus pneumoniae	5	2	0	7 (8.5)
Staphylococcus aureus	4	0	2	6 (7.3)
Total	70	6	6	82 (100)

Table 3. Age related frequency of bacterial isolation in the clinical samples

Bacteria Isolate	0-10 yrs	11-20 yrs	21-30yrs	31-40 yrs	41-50 yrs	51-60 yrs	61-70 yrs	70+yrs	Total
Klebsiella sp	2	4	5	6	9	3	0	3	32
Pseudomonas aeruginosa	3	2	3	3	0	3	0	2	16
Mycobacterium tuberculosis	0	0	4	3	2	2	0	0	11
Proteus sp	0	2	3	0	0	0	0	0	5
Escherichia coli	0	0	0	0	0	0	2	0	5
Streptococcus pneumoniae	0	0	2	0	2	3	0	0	7
Staphylococcus aureus	0	0	0	3	0	3	0	0	6
Total	5 (6)	8 (9.3)	17 (20.7)	15 (18.2)	13 (15.9)	14 (17)	2 (2.4)	3 (6)	82 (100)

NB: the two groups (21-30, 31-40) with the highest frequencies were compared using chi square (χ^2 =5.56, P<0.05)

Table 4. Gender related frequency of isolation of bacteria in clinical samples

Bacterial isolates	Male n(%)	Female n(%)	Total n(%)	Chi square	P-value
Klebsiella sp	20 (39.2)	12 (38.7)	32 (39)	0.002	0.962
Pseudomonas aeruginosa	11 (21.6)	5 (16.1)	16 (19.6)		0.775[a]
Mycobacterium tuberculosis	8 (15.7)	3 (9.7)	11 (13.4)		0.521[a]
Proteus sp	2 (3.9)	3 (9.7)	5 (6.1)		0.361[a]
Escherichia coli	2 (3.9)	3 (9.7)	5 (6.1)		0.361[a]
Streptococcus pneumoniae	5 (9.8)	2 (6.5)	7 (8.5)		0.705[a]
Staphylococcus aureus	3 (5.9)	3 (9.7)	6 (7.3)		0.668[a]
Total	51 (62.2)	31 (38.8)	82 (100)	9.756	0.002[b]

a=Fisher exact p-value
b=p<0.05 is significant

Table 5. Antimicrobial resistance pattern of bacterial isolates from the subjects

Antibiotics	Isolates						
	***Klebsiella* sp (n=32)**	***Pseudomonas aeruginosa* (n=16)**	***Proteus* sp (n=5)**	***E. coli* (n=5)**	***Streptococcus pneumoniae* (n=7)**	***Staphylococcus aureus* (n=6)**	**Total n (%) (n=71)**
Ofloxacin	1 (3.1)	4 (25)	0 (0)	2 (40)	0 (0)	0 (0)	7 (9.9)
Ciprofloxacin	3 (9.4)	3 (18.8)	1 (20)	3 (60)	0 (0)	1 (16.7)	11 (15.5)
Gentamicin	4 (12.5)	9 (56.3)	0 (0)	1 (20)	7 (100)	4 (66.7)	18 (25.4)
Ceftriaxone	10 (31.3)	12 (75)	1 (20)	0 (0)	0 (0)	1 (16.7)	24 (33.8)
Perfloxacin	0 (0)	8 (50)	0 (0)	2 (40)	0 (0)	1 (16.7)	11 (15.5)
Amoxicillin/clavulanic acid	24 (75)	13 (81.3)	4 (80)	2 (40)	2 (28.6)	1 (16.7)	46 (64.8)
Amoxicillin	26 (81.3)	14 (87.5)	3 (60)	3 (60)	3 (42.9)	4 (66.7)	53 (74.6)
Cotrimoxazole	29 (90.6)	NT	2 (40)	4 (80)	5 (71.4)	3 (50)	43/55(78.2)
Tetracycline	30 (93.8)	NT	4 (80)	4 (80)	3 (42.9)	3 (50)	44/55 (80)
Cloxacillin	NT	NT	NT	NT	5 (71.4)	5 (83.3)	10/13 (76.9)
Erythromycin	NT	NT	NT	NT	3 (42.9)	3 (50)	6/13 (46.2)
Chloramphenicol	NT	NT	NT	NT	6 (85.7)	2 (33.3)	8/13 (61.5)

NT= Not tested

Out of 82 (38.5%) pathogens detected in the samples, 70 (85.4%) were from sputum, six (7.3%) were from throat swab and six (7.3%) were from pleural aspirate. According to the report of Infectious Diseases Society of America (IDSA), collection of sputum samples was recommended for diagnostic of respiratory tract infections.[22] Also, Rozo and his associates[23] in their report stated that the sensitivity and specificity of gram stain from a good quality sputum specimen for diagnosis of *Streptococcus pneumoniae* were 57% and 82% respectively, and *Haemophilus influenzae* were 97% and 99% respectively. Although, Nagendra et al.[24] reported that sputum gram stain and culture are the most controversial among laboratory methods of diagnosing respiratory tract infections, the findings of this study still sees sputum as the most appropriate sample with the method used, especially culture samples diluted to ensure recovery of pathogens, provided the following conditions are met; proper collection of sputum and rapid transportation to the laboratory, adequate sampling of the purulent component of the sample, adequate staining technique and careful interpretation of results.[23] Other samples that could still be used are pleural aspirate and throat swab which is in line with the reports of other investigators.[25,26]

Among the bacterial species isolated, *Klebsiella* sp (n=32; 39%) was the commonest, followed by *Pseudomonas aeruginosa* (n=16; 19.6%), *Mycobacterium tuberculosis* (n=11; 13.4%), *Streptococcus pnuemoniae* (n=7; 8.5%), and *Staphylococcus aureus* (n=6; 73%). Reports from previous studies also indicated that *Klebsiella* sp was the most prevalent isolate in respiratory tract infection.[18,19]

The resistance patterns of the isolates indicated that the isolates (>60%) were commonly resistant to amoxicillin, cotrimoxazole and tetracycline. *Pseudomonas aeruginosa* isolates were commonly resistant to amoxicillin/clavulanic acid (n=13; 81.3%) and ceftriaxone (n=12; 75%) while isolates of *Streptococcus pneumoniae* were resistant to gentamicin (n=7; 100%), cloxacillin (n=5; 71.4%) and chloramphenicol (n=6; 85.7%). This pattern as seen in our study may be due to the unregulated use of antimicrobial in Nigeria. The least resistance was exhibited to ofloxacin (<10%), followed by ciprofloxacin (n=11; 15.5%) and perfloxacin (n=11; 15.5%). None of the isolates of *Proteus* sp, *Streptococcus pneumoniae*, and *Staphylococcus aureus* was resistant to ofloxacin. Likewise, none of the isolates of *Klebsiella,* was resistant to perfloxacin. This result indicates that pefloxacin, ofloxacin, and ciprofloxacin were the most effective antibiotics in the management of respiratory tract infections. Ciprofloxacin was recommended by Nester et al.[10] and Bayern [5] for treating RTIs. From the finding of his study, tetracycline, amoxillin and cotrimoxazole are not the drugs of choice for treating respiratory tract infections in the study environment.

5. CONCLUSION

In conclusion, the level of antibiotic resistance observed in this study is a serious public health problem and hence, underscores the need for prompt and proper diagnosis of respiratory tract infections. The reason for this observation may be due to indiscriminate and inappropriate use of drugs, adulteration of drugs and mutation of microorganisms, lack of infection control strategies and antibiotic stewardship programmes. There is a need for regular surveillance of bacterial infections and their antimicrobial susceptibility pattern in order to prevent the mobility and persistence of resistant pathogen in hospital and community settings.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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