



Prevalence and antibiotic sensitivity of bacterial agents involved in lower respiratory tract infections

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ABSTRACT

The prevalence and antibiotic sensitivity pattern of bacterial agents involved in Lower Respiratory Tract Infections (LRTI) was investigated. A total of 285 patients presenting with LRTI defined by a new or increasing cough, productive sputum, chest pain, fever, anorexia, haemoptysis, headache and throat ache were enrolled with their consent. The sputum specimen was cultured on the appropriate bacteriological media. Bacterial isolates were identified by standard laboratory and biochemical methods. Lower respiratory tract infection was found prevalent in 131 (46.0%) cases. Males 83 (63.4%) were found more at risk to LRTI than females, 48 (36.6%). Lower respiratory tract infection was found to be most prevalent in age group 40 – 49 years 39 (29.8%). *Streptococcus pneumoniae*, a Gram-positive bacteria, was identified as the most prevalent bacterial isolate 48 (34.3%) followed by *Klebsiella pneumoniae* 29 (20.7%), *Pseudomonas aeruginosa* 22 (15.7%) and *Staphylococcus aureus* 15 (10.7%). The overall antibiotic sensitivity test of the isolates showed ciprofloxacin 72 (51.4%), chloramphenicol 67 (47.9%) and gentamicin 39 (27.9%) as the most potent antibiotic against Gram-positive and Gram-negative isolates. High resistance was recorded for caftazidime, ceftizoxime, nalidixic acid, ampicillin, trimethoprim-sulfamethoxazole, cloxacillin and penicillin at 100% each. This study recorded a low percentage of sensitivity to the antibiotic agents tested.

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INTRODUCTION

An average person inhales at least eight microorganisms a minute or ten thousand each day (Prescott et al., 2005). A number of these microorganisms escape the human immune machinery and cause infections of the respiratory tract.

Respiratory tract infection is the leading cause of morbidity and mortality in critically ill patients in developing countries (Navneeth and Sandhya Belwadi, 2002; Kumari et al.,

2007), affecting the young and elderly almost equally. According to Gauchan et al. (2006), the top five respiratory diseases accounted for 17.4% of all deaths and 13.3% of all Disability-Adjusted Life Years (DALYs). Acute respiratory infections (ARI) and Tuberculosis were two of the six leading causes of death across all ages (World Health Organization, 2003). Also, out of the total acute respiratory diseases, 20–24% of all deaths are accounted for by Lower

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Respiratory Tract infection (LRTI) (Gauchen et al., 2006). It is notable that respiratory tract infections cause more disease and death than other infections in the United States, and there has been reported little change in mortality for more than five decades (Mizgerd, 2008).

Respiratory tract infections impose a serious economic burden on society, ranging from reduced output in workplaces to frequent prescription by physicians of antibiotics, even when the causative agents of infection is not bacteria (Jafari et al., 2009).

The causative agents of community-acquired lower respiratory tract infections are not well recognized, although it is traditionally taught that most are caused by viruses and atypical pathogens (Gauchen et al., 2006). A better understanding of the pathogens that cause these infections is recognized as a requirement which should allow a logical approach to treatment (Creer et al., 2006). There is the need, particularly in developing countries like Nigeria, for timely diagnosis of the major microbial causes of the respiratory infections in the community, and the administration of appropriate therapy based on the antibiotic susceptibility test of the causative agent in order to prevent further spread of the pathogen, which might otherwise lead to complications (Gauchen et al., 2006).

However, in recent years, there has been a dramatic rise in antibiotic resistance among respiratory pathogens (Imani et al., 2007). For instance, antibiotic resistance of Pneumococci to penicillin in the US before 1987 was < 1%, but, in 1997, the overall resistance was put at 48.8% (Imani et al., 2007). The consequences of increased drug resistance are far reaching since bacterial infection of lower respiratory tract is a major cause of death due to infectious disease (Kumari et al., 2007).

This study was undertaken to obtain information regarding prevalence of LRTI in individuals attending private diagnostic laboratories in Umuahia, Abia State, Nigeria,

as well as to have knowledge of the current antimicrobials sensitive to LRTI pathogens.

MATERIALS AND METHODS

The study was conducted on 285 patients attending private diagnostic laboratories in Umuahia, Abia State, Nigeria, between November, 2009 and May, 2010. The laboratory is Success Medical Diagnostic Laboratories and Blood Bank, Umuahia, Abia State. Samples of sputum for bacteriological culture and parasitological examination were collected after informed consent from patients presenting with LRTI (as defined by a new or increasing cough, productive sputum, chest pain, fever, anorexia, haemoptysis, headache and weight loss). Before culture, gram-stained smear of every specimen was first examined microscopically. In microscopic examination, sputum showing less than 10 squamous epithelial cells and more than 25 leucocytes or pus cells per low-power field confirmed the reliability of the specimen indicating that it was not contaminated with saliva.

The pathogens were isolated using suitable bacteriological media such as MacConkey Agar, Blood Agar and Chocolate Agar (Collee et al., 1996) supplemented with 8% sheep blood. Samples that showed pure growth of isolate in a count of $\geq 10^5$ cfu/ml of specimen after overnight incubation (at 37 °C for 18-24 hours) were identified microbiologically according to standard laboratory and biochemical method (Cheesbrough, 1984; Reisner et al., 1999). Antibiotic susceptibility was determined by the agar diffusion technique as described by Baker and Breach (1980). Isolates were considered as sensitive or resistant to an antibiotic according to the diameter of inhibition zone size interpretative chart (Chemical and Laboratory Standard Institute, 2006)

RESULTS

Out of the 285 sputum samples, 131 (46.0%) were positive for bacterial cultures,

154 (54.0%) gave no significant bacterial growth. Among the isolates, *Streptococcus pneumoniae* 48 (34.3%) was the most isolated organism, *Klebsiella pneumoniae* 29 (20.7%) was next, followed by *Pseudomonas aeruginosa* 22 (20.7%), *Staphylococcus aureus* 15 (10.7%), *Enterobacter* sp 10 (7.1%), coagulase-negative Staphylococci 9(6.4%), *Escherichia coli* 5 (3.6%) and *Proteus* sp 2 (1.4%) (Tables 1 and 2). Out of a total of 140 bacterial isolates, Gram-positive organism accounted for 72 (51.4%), while Gram-negative had 68 (48.6%).

Table 3 shows the prevalence of LRTI by Age and Gender. Out of 167 males enrolled in the study, 83 (63.4%) were positive for LRTI, while a total of 118 females enrolled gave 48 (36.6%) LRTI. The result shows that LRTI were more prevalent in males than in females. Age group, 40-49 years had the highest prevalence 39 (29.8%) of LRTI while, age group 10-19 years had the least 11 (8.4%).

The antibiotic sensitivity pattern of the isolates is shown in Tables 1 and 2. For Gram-negative isolates, the most effective antibiotic for *K. pneumonia* was ciprofloxacin 19 (65.5%), while it was resistant to ceftazidime

(100%) and ceftazoxime (100%). For *P. aeruginosa*, the most effective antibiotic was ciprofloxacin 8 (36.4%), while it was resistant to nalidixic acid (100%) and ampicillin (100%). The most effective antibiotic for *E. coli* was chloramphenicol 5 (100%), while it was resistant to ampicillin (100%). *Enterobacter* sp showed high sensitivity to ciprofloxacin 8 (80.0%), and high resistance to ceftizoxime (100%). Gentamycin 2 (100%) was highly effective for *Proteus* sp, while it was resistant to ampicillin (100%) and trimethoprim-sulfamethoxazole (100%).

Similarly, for Gram-positive isolates (Table 2), chloramphenicol 31 (64.6%) was the most effective antibiotic for *S. pneumoniae*, while the organism was resistant to cloxacillin (100%). The most effective antibiotic for *S. aureus* was gentamycin 11 (73.3%), while it showed resistance to ampicillin (100%), coxacillin (100%) and penicillin (100%). Gentamycin 5 (55.5%) and chloramphenicol 5 (55.5%) were the most effective antibiotic against coagulase-negative Staphylococci, while it showed high resistance to ampicillin (100%), cloxacillin (100%) and penicillin (100%).

Table 1: Antibiotic sensitivity pattern (%) of Gram-negative bacterial isolates from sputum samples.

Organisms	No. (%)	GN [#]	AMP	CP	CF	CFM	NA	C	CT	TSM
<i>K. pneumoniae</i>	29 (20.7)	13.8	6.9	65.5	6.9	0.0	3.4	37.9	0.0	34.5
<i>P. aeruginosa</i>	22 (15.7)	13.6	0.0	36.4	13.6	4.5	0.0	9.1	9.1	9.1
<i>E. coli</i>	5 (3.6)	60.0	0.0	40.0	20.0	20.0	40.0	100.0	20.0	20.0
<i>Enterobacter</i> sp	10 (7.1)	50.0	10.0	80.0	20.0	30.0	10.0	60.0	0.0	40.0
<i>Proteus</i> sp	2 (21.4)	100.0	0.0	50.0	50.0	50.0	50.0	50.0	50.0	0.0

[#]GN: Gentamycin (10 µg), AMP: Ampicillin (10 µg), CP: Ciprofloxacin (5 µg), CF: Ceftriaxone (5 µg), CFM: Ceftazidime (30 µg), NA: Nalidixic Acid (30 µg), C: Chloramphenicol (30 µg), CT: Ceftizoxime (30 µg), TSM: Trimethoprim-Sulfamethoxazole (30 µg).

Table 2: Antibiotic sensitivity pattern (%) of Gram-positive bacterial isolates from sputum samples.

Organisms	No. (%)	GN [#]	AMP	CP	CF	ER	CLX	PEN	C	TSM
<i>S. pneumoniae</i>	48 (34.3)	12.5	4.2	45.8	41.7	37.5	0.0	8.3	64.6	8.3
<i>S. aureus</i>	15 (10.7)	73.3	0.0	53.3	40.0	33.3	0.0	0.0	40.0	26.7
Coagulase-negative Staphylococci	9 (6.4)	55.5	0.0	44.4	33.3	22.2	0.0	0.0	55.5	33.3

[#]GN: Gentamycin (10 µg), AMP: Ampicillin (10 µg), CP: Ciprofloxacin (5 µg), CF: Ceftriaxone (5 µg), ER: Erythromycin (5 µg), CLX: Cloxacillin (5 µg), PEN: Penicillin (1 iu), C: Chloramphenicol (30 µg), TSM: Trimethoprim-Sulfamethoxazole (30 µg).

Table 3: Prevalence of LRTI by Age and Gender.

Age group (Years)	Male		Female		Total positive cases (%)
	No. of cases examined	Positive cases (%)	No. of cases examined	Positive cases (%)	
10 – 19	12	8 (72.7)	9	3 (27.3)	11 (8.4)
20 – 29	20	11 (73.3)	12	4 (26.7)	15 (11.5)
30 – 39	38	13 (56.5)	25	10 (43.5)	23 (17.6)
40 – 49	43	21 (53.8)	33	18 (46.2)	39 (29.8)
50 – 59	29	18 (69.2)	21	8 (30.8)	26 (19.8)
60 >	25	12 (70.6)	18	5 (29.4)	17 (13.0)
Total	167	83 (63.4)	118	48 (36.6)	131 (46.0)

% = Percentage.

DISCUSSION

Out of 285 sputum samples analyzed, 131 (46.0%) were positive for cultures leaving a greater negative result of 54 (54.0%). This finding is similar to studies carried out by Hosker (1994) and Gauchan et al. (2006), who also reported negative result of 60% and 56.4% respectively. This negative result may be attributed to viral or other etiological agents (Gauchan et al., 2006).

Out of the 140 bacterial isolates, Gram-positive organism were the highest of the isolates, accounting for 72 (51.4%), while Gram-negative had 68 (48.6%). Although, earlier study had shown Gram-negative bacterial isolates to be higher than Gram-positive bacterial isolates (Suyami and Shrestha, 1995; Gauchan et al., 2006), our findings correlates well with that of Shailaja et al. (2004).

Among the bacterial isolates, *S. pneumoniae* 48 (34.3%) was the most common isolate followed by *K. pneumoniae* 29 (20.7%), *P. aeruginosa* 22 (15.7%) and *S. aureus* 15 (10.7%). *S. pneumoniae* was also the predominant pathogen among the Gram-positive isolate whereas *K. pneumoniae* was predominant of Gram-negative isolate. Earlier study had also shown *S. pneumoniae* as the predominant Gram-positive isolates (MacFarlane et al., 1993; Shailaja et al., 2004; Taman et al., 2005). In a similar study by Tchamran (1997) on lung disease due to common bacteria, noted 81% of infections due to *S. pneumoniae*. Our findings also correlate with the study of Gauchan et al. (2006) and Jafari et al. (2009) who reported *Klebsiella* sp as the second predominant gram positive bacterial isolates.

In a study carried out by Dorobat et al. (2007) on the incidence and resistance pattern of pathogens from LRTIs; *Haemophilus influenzae* (34.65%) was the most prevalent gram negative organism followed by *Pseudomonas aeruginosa* (17.7%), *H. parainfluenza* (15.9%) and *K. pneumonia* (8.6%). The prevalent gram positive isolate was *Staphylococcus aureus* (54.1%) followed by *S. pneumonia* (45.9%). Jafari et al. (2009)

identified *Pseudomonas* sp [52(27.6%)] as the most prevalent bacterial isolate while *Klebsiella* sp [30(16%)] ranked third. Shailaja et al. (2004) had earlier reported *K. pneumonia* (32.26%) as the most prevalent bacterial isolate followed by *S. pneumoniae* (25.81%). They identified risk or susceptibility to infections with encapsulated organisms such as *S. pneumoniae* to be highest. The differences observed in the prevalence of bacterial isolates in studies elsewhere is attributable to age, season, the type of population at risk, and other factors (Collee and Watt, 1990).

This study showed males 83 (63.4%) to be at more risk to LRTI than females 48 (36.6%). Also, Humphrey et al. (2010), in their study of prevalence of pneumonia and lower respiratory tract infection, reported a high prevalence in males than females. According to Doddannavar (1885) as reported by Gauchan et al. (2006), the reason for the high risk in males of LRTI is attributable to decreased local immunity in the respiratory tract due to smoking, use of tobacco, alcohol consumption etc.

This study also recovered the highest prevalence 39 (29.8%) of LRTI among age group 40-49years. The least prevalence, 11 (8.4%) was among age group 10-19 years. Prevalence decreased from age group 40-49 years. Although, similar studies have reported increased pneumococcal infections in patients 55 years and above, who also have an increasing incidence of LRTI as they age (MacFarlane et al., 1993) due to decreased immune system resulting in malnutrition or degenerative diseases such as diabetic mellitus, uremia, emphysema etc.

The sensitivity tests (Table 1 and 2) indicated that the isolates were resistant to one or more antibiotic, although generally, a low percentage of the isolates were sensitive to the antibiotic tested. The result of the sensitivity test indicates that Gram-positive and Gram-negative isolates showed highest sensitivity to ciprofloxacin, chloramphenicol and gentamycin, while high resistance was also recorded for antibiotics such as caftazidime,

ceftizoxime, nalidixic acid, ampicillin, nalidixic acid, ampicillin, trimethoprim-sulfamethoxazole, cloxacillin and penicillin. This implies that treatment of possible infection due to these organisms may not be feasible and would require a new antibiotic which are not commonly available. This observation poses a serious public health problem.

The pattern of antibiotic resistance recorded in this study among *P. aeruginosa*, *K. pneumoniae* and *E. coli* isolates is consistent with results obtained from other developing countries (Gauchan et al., 2006; Kumari et al., 2007). Although *P. aeruginosa* has been shown to be resistant to many antimicrobial agents, ciprofloxacin was shown to be the most potent quinolone against the pathogen (Walker, 1999), which is consistent with our findings.

However, Lyon et al. (2001) identified high rates of resistance of *S. Pneumoniae* isolates to various fluoroquinolones in their study in Hong Kong. Dobobat et al. (2007) reported a low resistance of *K. pneumoniae* to ciprofloxacin (7.8%) compared to our finding of 34.5% resistance. Fluoroquinolone resistance was also identified in United Kingdom, France and Spain (Doern et al., 1996; Schito et al., 2000). Although our data from this study identified ciprofloxacin (a fluoroquinolone) as one of the most potent antibiotic against LRTI pathogens, its resistance recorded in our study was higher compared to earlier reports. For example, in an earlier study by Imani et al. (2007), resistance of *S. Pneumoniae* to ciprofloxacin was 29.4%, while ours was 54.2%. According to Imani et al. (2007), attention has been drawn to decreased susceptibility of *S. Pneumoniae* to fluoroquinolones, perhaps reflecting increased use of this class of antibiotic.

Resistance to penicillin and ampicillin by respiratory tract pathogens reported by several researchers is of particular concern. This implies that these drugs are no longer feasible in the treatment of most bacterial infections. For example, Imani et al. (2007)

reported a 100% resistance of *H. influenzae* and *Morexella catarrhalis* to penicillin and ampicillin, while *S. Pneumoniae* showed 94.1% resistance. Dorobat et al. (2007) reported high resistance (13.4%) of *S. Pneumoniae* strains to penicillin, with 39.3% intermediate resistance. Earlier, in 1998, the Asian Network for Surveillance of Resistant Pathogens (ANSORP) study reported penicillin resistance in Korea almost 80% of all isolates, with a similar result in Nagasaka and Japan (Song et al., 1999). In France, the prevalence of penicillin-resistant *S. Pneumoniae* was 53.3% of submitted isolates in 1992 (Imani et al., 2007). At the same time, Germany had low resistance (7.2%) (Schito et al., 2000). Similarly, this study recorded high resistance to penicillin and ampicillin for almost all isolate.

Low levels of chloramphenicol resistance among LRTI pathogens was reported by Dorobat et al. (2007). In comparison, our study recorded high levels of pneumococcal isolates resistance to chloramphenicol, but more feasible than most antibiotic tested.

Differences in the prevalence of antimicrobial resistance in countries may be due to several factors such as different patterns of antimicrobial usage, which lead to variable selective pressure on resistance (Wilson, 2001). Other factors include distribution of specific serotypes and the spread of resistant clones within certain regions (Imani et al., 2007).

The pattern of antibiotic resistance in *S. aureus* was observed to be similar to that of coagulase-negative staphylococci (Table 2). The possible explanation of the antibiotic resistance pattern among the isolates in recent years is not unconnected to indiscriminate and promiscuous use. Frequent prescription by physicians of antibiotics, even when the causative agents of infection is not clear (Jafari et al., 2009) further aggravates the problem of antibiotic resistance. The use of suboptimal and long duration regimens in the case of *S. pneumoniae* increases the opportunity for acquisition and/or

amplification of resistant strains (Gauchan et al., 2006).

Conclusion

This study revealed that there has not been any significant change in the pattern of bacterial pathogens involved in LRTI. Also, the age group at risk of LRTI remains more or less the same. The level of antibiotic resistance observed in this study is a serious public health problem and hence, brings to light the need for timely and proper diagnosis of the major microbial causes of the respiratory infections, in order to administer the appropriate therapy based on antibiotic susceptibility test of the causative agent. Mass literacy campaign on the need to seek medical attention when necessary and judicious use of antibiotics is recommended to help check the emergence of drug resistance pathogens. There is the need for further research on antibiotic resistance using different antibiotic with a view to identifying one with which LRTI pathogens are almost 100% susceptible to.

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