

ORIGINAL RESEARCH ARTICLE

Antibiotic Susceptibility Profile of Respiratory Pathogens Obtained at Tertiary Care Hospital from Western Nepal

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ABSTRACT

The prevalence and drug resistance of the respiratory pathogens is increasing gradually in Nepal. However, their detail study is rare in the western region of Nepal. Hence, this study was carried out to know the incidence and antibiotic susceptibility profile of the respiratory pathogens obtained at a tertiary care center located at Pokhara. 139 pathogens were isolated from 460 clinical samples included. Significant pathogens were Gram-negative bacteria 94 (67.62%), followed by 28 (20.15%) *Candida*, and Gram-positive isolates 17 (12.23%). The growth rate was significantly higher for sputum samples in comparison to throat swabs. *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter* spp were significant Gram-negative isolates while *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes* were Gram-positive pathogens. Sensitivity rate was higher for colistin and imipenem among Gram-negative isolates while lower sensitivity was for cefepime. Vancomycin was effective against all tested Gram-positive isolates while erythromycin and ciprofloxacin were less effective.

Keywords: respiratory pathogens, western Nepal, *Pseudomonas aeruginosa*, *Acinetobacter* spp, *Klebsiella pneumoniae*, *S. aureus*.

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Introduction

Infection of the respiratory tract is a significant cause of mortality as well as morbidity among the elderly and young people of Nepal [1]. Management of these infections is more complex in developing countries due to the lack of the proper identification of pathogens and their suitable treatment [2]. The causative agents of the respiratory infections are not detected in many cases hence physicians depend on clinical manifestations for the diagnosis [3]. *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* are the significant pathogens causing respiratory infections [4, 5]. In addition to this, pathogens like *Staphylococcus aureus*, *Acinetobacter*, and *Pseudomonas aeruginosa* are also frequently isolated from respiratory specimens [6, 7].

Polymicrobial respiratory infections by two or more bacteria, two or more viral pathogens, and mixed viral and bacterial pathogens also have been reported [4, 5]. Pneumonia and infection of

lower respiratory tract are the causes of more than 4 million deaths per year and this problem is more frequent in middle-and low-income nations [8]. Drug resistance among these pathogens has been increasing as there are multiple reports of multidrug resistance among various respiratory pathogens and many of them are not susceptible to several routine antibiotics [4, 9]. Though most of the bacterial respiratory infections are treatable, the huge death is due to a lack of proper preventive measures and unavailability of healthcare facilities [8]. Several reports are indicating a gradual increase in antibiotic resistance among many bacterial pathogens responsible for respiratory diseases [10, 11].

The imprudent use of antibiotics for treating these infections has resulted in a very rapid increase in drug resistance of the respiratory pathogens [12]. Though various pathogens [13] and risk factors [14] are associated with



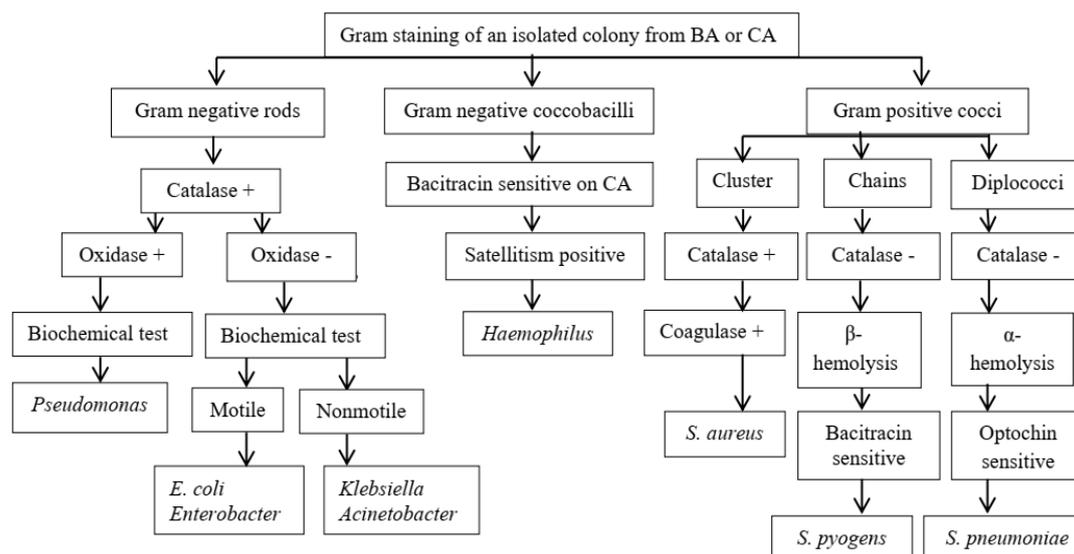


Figure 1: Identification chart for the pathogens isolated from respiratory samples.

respiratory infections, their detail study is very limited in this region. Surveillance study on respiratory pathogens was imminent in this part of the nation. Hence this study was done to assess the prevalence, distribution and drug resistance profile of the respiratory pathogens isolated at a tertiary care center of western Nepal.

Materials and Methods

Study site and duration

The study site was Manipal Teaching Hospital, a 750-bed multi-specialty tertiary care hospital located at Pokhara, Nepal. The study was conducted for a period of six months (July 2016 to January 2017) at the Microbiology laboratory of the hospital.

Sample collection

Samples were collected from patients with clinical symptoms of respiratory tract infection as indicated by physicians. For the collection of sputum samples, patients were given various instructions on how to collect a sputum sample correctly. Clean, well-labeled, wide-necked, dry and leak-proof screw-cap container was provided to the patients for sputum collection. The sample was accepted only if it was sputum but samples containing saliva, nasal secretions, mucus, etc. were not analyzed. In the case of unacceptable samples, repeated samples were requested. Throat sample was collected by trained personals. The patient was first allowed

to sit comfortably in a good light and using a tongue depressor the throat was observed for any swelling, redness, pus, ulcerations, exudates, and presence of the membrane. A sterile cotton swab was used to collect a sample of the infected throat. Special care was taken not to contaminate the swab with saliva and placed it into a sterile container.

Macroscopic and microscopic examination of the sample

Appearance, as well as presence or absence of blood in the given sputum, was observed and noted. Similarly, gram staining was performed from both sputum and throat swabs to observe pus cells and bacteria.

Isolation and identification

Respiratory tract samples obtained from both admitted and outpatients visiting the hospital were included in this study. Soon after collection, all the specimens were cultured on chocolate agar and 5% sheep blood agar. The inoculated plates were then incubated on candle jar for 18-24 hours. Optochin and bacitracin discs were placed on the primary inoculation for the presumptive screening of *S. pneumoniae* and *H. influenzae* as well as *S. pyogenes* respectively. Identification of the pure culture was carried out by observing colony characteristics and gram staining followed by oxidase test, catalase test, coagulase test, urease test, TSI test, and IMViC tests.

Antibiotic susceptibility test

Kirby-Bauer disc diffusion method was used using Muller Hinton agar (Hi-Media, India). Clinical and Laboratory Standards Institute (CLSI) guidelines were used for the interpretation of the results [15]. 0.5 Mac Farland suspension of the isolates were used for inoculation. Amikacin (30µg), Azithromycin (15µg), Cefepime (30µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Colistin, (10µg) Co-Trimoxazole (25µg), Imipenem (10µg), Piperacillin/ Tazobactam (100µg/10), Vancomycin (30µg), Levofloxacin (5µg), Teicoplanin (30µg), and Erythromycin (15µg) were used for the test. *E. coli* ATCC 25922 was used as a control organism for Gram-negative isolates while *Staphylococcus aureus* ATCC 25923 was used as a control organism for Gram-positive isolates during this test.

Results

Among 460 samples, 139 (30.2%) showed the growth of respiratory pathogens. The incidence of the pathogens was higher from the sputum sample in relation to the throat sample (Table 1). *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, and *Candida* spp. were the common pathogens isolated followed by *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Enterobacter* spp. *Streptococcus pyogenes* were isolated only from throat swab while all gram-negative isolates were only from the sputum sample. Only *Candida* was isolated from both of the samples (Table 2).

Table 1: Total cases and types of samples

Samples	Growth positive (%)	Growth negative (%)	Total (%)
Sputum	134 (29.13%)	299 (65%)	433 (94.13%)
Throat swab	5 (1.07%)	22 (4.8%)	27 (5.87%)
Total	139 (30.2%)	321 (69.8%)	460 (100%)

Vancomycin was the most effective drug for all three Gram-positive pathogens as all of these isolates were sensitive to vancomycin. Teicoplanin and clindamycin were also highly

Table 2: Distribution of Gram-positive cocci, Gram-negative bacilli and *Candida* spp. in throat swab and sputum samples

Microorganisms	Samples		Total (%)
	Throat swab (%)	Sputum (%)	
<i>Pseudomonas aeruginosa</i>	0	31(22.3)	31 (22.3)
<i>Acinetobacter</i> spp	0	26 (18.7)	26 (18.7)
<i>Klebsiella pneumoniae</i>	0	23 (16.55)	23 (16.55)
<i>Staphylococcus aureus</i>	0	9 (6.47)	9 (6.47)
<i>Escherichia coli</i>	0	8 (5.76)	8 (5.76)
<i>Haemophilus influenza</i>	0	4 (2.88)	4 (2.88)
<i>Streptococcus pneumoniae</i>	0	4 (2.88)	4 (2.88)
<i>Streptococcus pyogenes</i>	4 (2.88)	0	4 (2.88)
<i>Enterobacter</i> spp	0	2 (1.44)	2 (1.44)
<i>Candida</i> spp.	1 (0.72)	27 (19.42)	28 (20.14)
Total	5 (3.6)	134 (96.4)	139 (100)

active against *S. aureus* and *Streptococcus pyogenes* respectively while other drugs were less effective as there was resistance ranging from 22 to 100% for antibiotics other than vancomycin, teicoplanin, and clindamycin (Table 3). Colistin was the most effective drug for treating *Pseudomonas aeruginosa* and *Acinetobacter*. Least drug resistance was noted on *Enterobacter* while the highest resistance was observed on *Acinetobacter*. Resistance towards all common antibiotics was observed in all Gram-negative pathogens excluding *Enterobacter* with varying percentages (Table 4).

Discussion

Nearly one-third of the samples were positive for the respiratory pathogens. Findings of the Shrestha et al (2005) also reported that 31% of the sputum samples were positive for pathogens [16]. Among the isolated pathogens Gram-negative isolates were more prevalent than Gram-positive pathogens.

Table 3: Antimicrobial susceptibility profiles of Gram-positive isolates

Antibiotics used	Pathogens					
	<i>Staphylococcus aureus</i> (n=9)		<i>Streptococcus pyogenes</i> (n=4)		<i>Streptococcus pneumoniae</i> (n=4)	
	Sensitive No. (%)	Resistant No. (%)	Sensitive No. (%)	Resistant No. (%)	Sensitive No. (%)	Resistant No. (%)
Amikacin	7 (77.8)	2 (22.2)	3 (75)	1 (25)	-	-
Ciprofloxacin	3 (33.3)	6 (66.7)	-	-	-	-
Clindamycin	2 (22.2)	7 (77.8)	4 (100)	0	2 (50)	2 (50)
Co-Trimoxazole	-	-	-	-	1 (25)	3 (75)
Erythromycin	2 (22.2)	7 (77.8)	0	4 (100)	2 (50)	2 (50)
Levofloxacin	-	-	0	4 (100)	3 (75)	1 (25)
Teicoplanin	9 (100)	0	-	-	-	-
Vancomycin	9 (100)	0	4 (100)	0	4 (100)	0

Table 4: Antimicrobial susceptibility profiles of Gram-negative bacilli

Antibiotics Used	Pathogens					
	<i>Pseudomonas aeruginosa</i> (n=31)	<i>Acinetobacter spp</i> (n=26)	<i>Klebsiella pneumoniae</i> (n=23)	<i>E. coli</i> (n=8)	<i>Haemophilus influenzae</i> (n=4)	<i>Enterobacter spp</i> (n=2)
	S (%)	S (%)	S (%)	S (%)	S (%)	S (%)
Amikacin	26 (83.9)	8 (30.8)	15 (65.2)	8(100)	-	2 (100)
Azithromycin	-	-	-	-	2 (50)	-
Cefepime	7 (22.6)	6 (23.1)	6 (26.1)	0	-	2 (100)
Ceftriaxone	-	-	-	-	3 (75)	-
Ciprofloxacin	22 (71)	8 (30.8)	10 (43.5)	2 (25)	4 (100)	2 (100)
Colistin	31 (100)	26 (100)	-	-	-	-
Co-Trimoxazole	-	-	8 (34.8)	5 (62.5)	2 (50)	2 (100)
Imipenem	23 (74.2)	9 (34.6)	21 (91.3)	7(87.5)	-	2 (100)
Piperacillin/Tazobactam	13 (42)	5 (19.2)	7 (30.4)	3 (37.5)	-	2 (100)

A very high incidence of Gram-negative pathogens among respiratory samples are also reported earlier [2, 12, 16]. Gram-negative isolates like *Pseudomonas aeruginosa*, *Acinetobacter spp.*, *Klebsiella pneumoniae* were predominant. These findings were in accordance with the previous studies [13, 17]. Similarly, *Staphylococcus aureus* was the major Gram-positive pathogens.

Pathogens like *Streptococcus pneumoniae*, *Streptococcus pyogenes* were also isolated from the respiratory samples. The predominance of *Staphylococcus aureus* among Gram-positive pathogens followed by *Streptococcus spp.* is common from respiratory samples [1, 3, 18]. *Candida* isolates were also the major cause of

respiratory infections as indicated by previous authors [3, 19].

Vancomycin was the most reliable treatment option for all of the gram-positive pathogens isolated. The high sensitivity of vancomycin against *S. aureus* and *Streptococcus* spp isolated from respiratory tract infection is found elsewhere [18, 19, 20]. More than 66% of *S. aureus* were resistant to Ciprofloxacin, Clindamycin, and Erythromycin. Increasing resistance of *S. aureus* towards these drugs is common globally [18, 20]. All *Streptococcus pyogenes* isolated in this study were sensitive to Clindamycin and Vancomycin while they were absolutely resistant to Levofloxacin and Erythromycin. Very similar antibiotic sensitivity patterns of *Streptococcus pyogenes* are reported earlier [21, 22]. The majority of *Streptococcus pneumoniae* were resistant against Co-Trimoxazole, Clindamycin, and Erythromycin. An elevated level of resistance among *Streptococcus pneumoniae* obtained from sputum and other samples are reported from different countries [23].

Colistin resistance was not observed in *Pseudomonas aeruginosa* and *Acinetobacter* spp. indicating that this drug is still effective as a last resort. There are many reports of absolute colistin sensitivity among gram-negative respiratory pathogens [24, 25]. However, there is a report of colistin resistance among multidrug-resistant *Acinetobacter* spp isolates obtained from ICU patients suffering from respiratory tract infection in Nepal [26]. Imipenem and amikacin resistance was less in gram-negative isolates in comparison to other antibiotics like Cefepime, Ciprofloxacin, Piperacillin/ Tazobactam. Less resistance is detected in common respiratory gram-negative pathogens against Imipenem and amikacin [24, 27]. Drug resistance was higher in *Acinetobacter* spp among Gram-negative pathogens. Highly elevated antibiotic resistance among *Acinetobacter* isolated from respiratory tract infection is reported previously in Nepal [17, 26]. We also noticed drug resistance among *Haemophilus influenzae* as some isolates were not sensitive to Azithromycin, Ceftriaxone, and Co-Trimoxazole. Antibiotic-resistant *Haemophilus influenzae* causing respiratory tract infection is not new [2].

Conclusion

Pseudomonas aeruginosa, *Acinetobacter* spp., *Klebsiella pneumoniae*, *Candida*, and *S. aureus* were the major isolates causing respiratory infection. Many of these isolates were resistant to various routine drugs. To prevent the further increase of antimicrobial resistance among the respiratory isolates antibiotic susceptibility testing must be done before therapy.

Author Contributions

DS and SPD designed the research proposal, DS performed the lab works, DRB, BKS, and KG supervised the work, AP, SPD and DG wrote the article. All the authors read, revised, and finalized the manuscript.

Acknowledgment

The authors are thankful to all the members of the Microbiology laboratory of Manipal Teaching Hospital for their continuous support during this work as well as to the patients for providing samples for this research.

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