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Bacteriological profile and antimicrobial susceptibility pattern of sputum samples in patients presenting to the pulmonology ward of a tertiary care hospital of Peshawar

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ABSTRACT

Introduction: Pathogens causing respiratory infections keep changing, and a wide array of pathogens are implicated as causative agents. This diversity has imposed a challenge for clinicians to establish a specific etiological diagnosis and initiate the most suitable antimicrobial therapy.

Objective: To document the most common bacteriological organisms detected from sputum culture in patients presenting to the Pulmonology ward of Rehman Medical Institute (RMI) and determine their antimicrobial sensitivity patterns.

Materials & Methods: Sputum culture of 50 patients presenting to the pulmonology department from 2016-2017 were collected in the lab after informed consent. Anti-microbial susceptibility testing of microbial isolates was performed according to the guidelines issued by the National Committee for Clinical Laboratory Standards. Different antimicrobials were used for different pathogens based on the type of resistance and availability. Growth of the microbe was inhibited around discs containing antimicrobials agents to which it was susceptible but not around those to which it was resistant.

Results: The most commonly isolated pathogen was *Pseudomonas* species (n=12) followed by *Escherichia coli* (n=10), and *Staphylococcus aureus* (n=7). Gram negative bacteria made up 78% and gram positive bacteria 22% of the pathogens. *Pseudomonas* species was susceptible to most of the drugs and showed resistance towards Aztreonam (36.36%) and Ciprofloxacin (33.33%). It showed (100%) susceptibility to colistin and meropenem. *Escherichia coli* was 100% resistant towards Amoxicillin, Ampicillin, Ciprofloxacin, Cefotaxime, and Doxycycline. Least resistance was seen to Piperacillin/Tazobactam (55%), Cefoxitin (33.33%), Imipenem (20%) and Gentamicin (25%).

Conclusions: Varying patterns of resistance of pathogens towards commonly used antimicrobial agents existed, with most pathogens being resistant to third generation Cephalosporins and extended spectrum Penicillins.

Keywords: Sputum, Anti-bacterial agents, *Pseudomonas*, *Streptococcus pneumoniae*, Imipenem, Aztreonam, Pulmonary Medicine.

The authors declared no conflict of interest. All authors contributed substantially to the planning of research, data collection, data analysis, and write-up of the article, and agreed to be accountable for all aspects of the work.

INTRODUCTION

Sputum culture is a diagnostic tool to detect bacteria and fungi present in the sputum. Its role to diagnose bacterial or fungal respiratory infections remain controversial because it is difficult to obtain good quality samples. The reliability of the culture is affected due to contamination of the sputum by the flora of the upper airway and it has a low diagnostic value. These limitations impact treatment options and decisions.[1] Rapid and specific tests to detect agents of bacterial pneumonias are not always present and cultures take 24 hours to yield. Therefore, initially, empirical antibiotic therapy is chosen.[2]

Pathogens causing respiratory infections are changing. For example, forty years ago, *Streptococcus pneumoniae* accounted for most of the community acquired pneumonia. Now, a wide array of pathogens is implicated as causative agents. These include *Mycoplasma* pneumonia in youngers adults and in older adults *Legionella* species, *Staphylococcus Aureus*, *Hemophilus Influenza* and gram negative bacilli have been seen as etiological agents of community acquired pneumonia.[3] The diversity in pathogenic agents have imposed a challenge on clinicians to establish a specific etiological diagnosis to initiate the most suitable antimicrobial therapy.

Antibiotic sensitivity pattern varies day by day and is rapidly emerging due to over the counter sales and increasing abuse of antibiotics which has posed a threat to the appropriate management of Respiratory tract infections. A report on antimicrobial threats by the Center for Disease Control (CDC) reported the considerable economic burden resistant infections come with. Infections resistant to first and second line treatments are costlier to treat and prolong hospital stay. The cost of antimicrobial resistant infections on the US economy was estimated to be as high as 20 billion USD.[4]

The present study was conducted to determine the antibiotic resistance pattern of different organisms that cause respiratory infections by examining the sputum cultures of the patients and to assess the susceptibility of bacteria in order to highlight the ones that are concerning and pose a threat to healthcare practices.

MATERIALS & METHODS

Sputum cultures of 50 patients presenting to pulmonology department of RMI over the period of two years (2016-2017) were analyzed. Age, sex and symptoms were noted. Clearance was obtained from the institute's ethics committee and consent was taken from patients.

The standard process was adopted for collection of sputum and blood for culture. Expectorated sputum samples were collected at the hospital's lab before starting antibiotic therapy. Patients were requested by the nurse or lab technician to produce sputum. Samples were gram stained and the suitable ones were accepted for culture. Specimens were incubated overnight in an atmosphere containing 5% CO_2 in air in standard fashion on the following agars: sheep blood, chocolate, and eosin-methylene blue. Standard microbiological techniques were used to identify organisms.[5] Viridans group *Streptococci*, coagulase-negative *Staphylococci*, and some *Neisseria* species were considered as part of normal respiratory flora. Beta-hemolytic *Streptococci, Staphylococcus aureus, Streptococcus pneumoniae, Hemophilus influenza, Neisseria meningitis*, and gram negative bacilli were considered to be potential pathogens.

Each bacterial isolate was prepared for susceptibility testing procedures by following the guidelines published by Clinical Laboratory Standards Institute.[6] Different antimicrobials were used for different pathogens based on the type of resistance and availability. Growth of the microbe was inhibited around discs containing antimicrobials to which it was susceptible but not around those to which it was resistant.

RESULTS

Sputum of 50 patients were analyzed for this study out which one yielded *Streptococcus viridans*, since it is a part of normal flora of the respiratory tract, and was not tested for antimicrobial susceptibility. Out of the 50 patients, 17 were females and 33 were males. Age of the patients ranged from 1 year to 85 years. The most commonly isolated pathogen was *Pseudomonas* species (n=12) followed by *Escherichia coli* (n=10), and *Staphylococcus aureus* (n=7). Gram negative bacteria made up 78% of the pathogens and 22% were gram positive bacteria.

Table 1: Profile of bacteria isolated from sputum (n = 49).

Bacterial Isolates category	Frequency
Gram negative	(39)
Pseudomonas species	12
Escherichia coli	10
Acinetobacter species	5
Klebsiella pneumonia	4
Enterobacter species	2
Stenotrophomonas maltophilia	2
Serratia liquefaciens	2
Moraxella catarrhalis	1
Citrobacter species	1
Gram positive	(11)
Staphylococcus aureus	7
Streptococcus pneumoniae	2
Beta hemolytic streptococci	1
Streptococcus viridans	1

Table 2 summarizes the antimicrobial susceptibility patterns of the pathogens isolated. *Staphylococcus* showed most resistance to Ampicillin (100%), Amoxicillin (100%), Azithromycin (85.71%) and Clarithromycin (100%). Resistance to Cefoxitin (71%) Ceftriaxone (66.67%) and Imipenem (60%) was seen but some isolates were susceptible. *Streptococcus pneumoniae* was resistant to Ceftriaxone (100%), Clindamycin (100%) and Erythromycin (100%) All the antimicrobials tested against *Streptococcus pyogenes* were effective in eliminating the microbe. The pathogen was completely susceptible to Ampicillin, Cefotaxime, Ceftriaxone, Erythromycin and Linezolid.

Table 2: Antimicrobial resistance (%) and its pattern of gram-positive bacterial isolates from sputum of patients presenting to RMI pulmonology ward

Antimicrobial agent	Staphylococcus (n=7)	S. pneumonia (n=2)
Amikacin	2 (28.57%)	
Amoxicillin	6 (100.00%)	
Amoxicillin / Clavulanic acid	3 (75.00%)	
Ampicillin	7 (100.00%)	
Ampicillin/Sulbactam	4 (100.00%)	
Azithromycin	6 (85.71%)	
Cefaclor	4 (66.67%)	
Cefoperazone / Sulbactam	3 (75.00%)	
Cefazolin	4 (66.67%)	
Cefotaxime		
Cefoxitin	5 (71.43%)	
Cefpodoxime	3 (60.00%)	
Ceftizoxime	3(60.00%)	
Ceftriaxone	4 (66.67%)	*1 (100%)*
Cefuroxime	4 (66.67%)	
Cephalexin	4 (66.67%)	
Chloramphenicol	0 (0%)	0 (0%)
Ciprofloxacin	4 (66.67%)	
Clarithromycin	6 (100.00%)	
Clindamycin	3 (50%)	*1 (100%)*
Co-trimoxazole	1 (20%)	
Doxycycline	2 (28.57%)	
Erythromycin	5 (83.33%)	1 (100%)
Floxacillin	2 (50.00%)	
Fosfomycin	0 (0%)	
Gentamicin	1 (14.29%)	0 (0%)
Imipenem	3 (60.00%)	
Levofloxacin	1 (50.00%)	*1 (50%)*
Linezolid	0 (0%)	0 (0%)
Meropenem	2 (100.00%)	
Minocycline	1 (20.00%)	
Moxifloxacin	1 (100.00%)	
Penicillin	3 (100.00%)	0 (0%)
Piperacillin	2 (66.67%)	
Piperacillin / Tazobactam	3 (75.00%)	
Rifampicin	1 (25.00%)	0(0%)
Vancomycin	0 (0%)	0 (0%)

Table 3 demonstrates the antimicrobial susceptibility patterns of the gram negative bacteria isolated. Most common pathogen yielded was *Pseudomonas* species (n=12). The isolates were susceptible to most of the drugs and showed resistance towards Aztreonam (36.36%) and Ciprofloxacin (33.33%). It showed 100% susceptibility to Colistin and Meropenem and only 10% of the isolates were resistant to Piperacillin/Tazobactam.

Escherichia coli showed an interesting antimicrobial susceptibility pattern; 100% resistance was seen towards Amoxicillin, Ampicillin, Ciprofloxacin, Cefotaxime and

Doxycycline. Least resistance was seen to Piperacillin / Tazobactam (55%), Cefoxitin (33.33%), Imipenem (20%) and Gentamicin (25%). Ciprofloxacin (22.22%), Co-Trimoxazole (33.33%) and Aztreonam (25%) were effective in eliminating some strains but most of the strains of *Escherichia coli* were resistant to these antimicrobial agents. *Acinetobacter* species showed 100% resistance to almost all the antimicrobials which included Amikacin, Ampicillin, Doxycycline and Gentamicin. It was susceptible to only a few drugs such as Co-trimoxazole (33.33%) and Minocycline (60%). *Klebsiella pneumonia* also showed 100% resistance to many antimicrobials which included Ampicillin/Sulbactam, Aztreonam, Cefipime and Ceftazidime. It was however susceptible to Amikacin (75%), Ciprofloxacin (75%) and Colistin/Polymixin B (100%).

Stenotrophomonas maltophilia and *Serratia liquefaciens* were also seen on cultures. Amikacin, Amoxicillin/Clavulanic Acid, Ampicillin, Cefotaxime and Ceftazidime were completely ineffective in eliminating *Stenotophomonas maltophilia* but the pathogen was susceptible to Ciprofloxacin (100%), Co-Trimoxazole (100%) and Minocycline (100%). Imipenem (100%) and Piperacillin / Tazobactam (100%) were the only antimicrobials which successfully eliminated isolates of *Serratia liquefaciens*. The microbe was resistant to Cefipime (100%), Cefuroxime (100%) and Levofloxacin (100%).

Table 3: Gram negative bacteria and their pattern of resistance

Antimicrobial agent	Pseudomonas species (n=12)	Escherichia coli (n=10)	Acinetobacter (n=5)	Klebsiella pneumoniae (n=4)	Enterobacter species (n=2)	Stenotrophomonas maltophilia (n=2)	Serratia liquefaciens (n=2)
Amikacin	1 (8.33%)	1 (10%)	4 (100%)	1 (25%)	0 (0%)	1 (100%)	1 (50%)
Amoxicillin		*3 (100%)*	*1 (100%)*		*1 (100%)*	*1 (100%)*	*1 (100%)*
Amoxicillin / Clavulanic acid		3 (100%)		*1 (100%)*	1 (100%)	*1 (100%)*	1 (100%)
Ampicillin		9 (100%)	3 (100%)	*3 (100%)*	1 (100%)	2 (100%)	1 (100%)
Ampicillin / Sulbactam		*3 (75%)*	3 (75%)	*4 (100%)*	*0 (0%)*		*1 (100%)*
Aztreonam	4 (36.36%)	6 (75%)		4 (100%)			
Cefazolin		*3 (100%)*	*1 (100%)*		*2 (100%)*		
Cefipime	1 (9.09%)	9 (90%)		4 (100%)	2 (100%)	2 (100%)	*2 (100%)*
Cefixime		*2 (100%)*		*1 (100%)*			
Cefoperazone / Sulbactam			1 (100%)				
Cefotaxime		10 (100%)	2 (100%)	*3 (100%)*	1 (50%)	*2 (100%)*	1 (100%)
Cefoxitin		1 (33.33%)	2 (100%)		1 (100%)	1 (50%)	1 (100%)
Ceftazidime	3 (30%)	9 (100%)	1 (50%)	4 (100%)	2 (100%)	2 (100%)	2 (100%)
Ceftriaxone		2 (100%)	2 (100%)	*1 (100%)*		1 (100%)	
Cefuroxime		*7 (100%)*	*1 (100%)*	*2 (100%)*	*2 (100%)*	*1 (100%)*	*2 (100%)*
Chloramphenicol							
Ciprofloxacin	4 (33.33%)	7 (77.78%)	5 (100%)	1 (25%)	0 (0%)	0 (0%)	*1 (50%)*
Colistin	0 (0%)		0 (0%)				
Colistin / Polymyxin B		0 (0%)		*0 (0%)*			0 (0%)
Co-trimoxazole		4 (66.67%)	1 (33.33%)	*2 (100%)*	0 (0%)	0 (0%)	2 (100%)
Doxycycline		4 (100%)	3 (100%)	*2 (100%)*		*1 (50%)*	*1 (50%)*
Erythromycin							
Gentamicin	2 (16.67%)	2 (25%)	4 (100%)	2 (50%)	1 (100%)	0 (0%)	*1 (50%)*
Imipenem	0 (0%)	2 (20%)	4 (80%)	0 (0%)	0 (0%)	1 (50%)	*0 (0%)*
Levofloxacin	1 (12.5%)	6 (75%)		1 (33.33%)	0 (0%)	*0 (0%)*	1 (100%)
Meropenem	0 (0%)	2 (66.67%)			0 (0%)		0 (0%)
Norfloxacin	1(100%)						
Minocycline		*7 (77.78%)*	3 (60%)	*3 (100%)*	*2 (100%)*	*0 (0%)*	*1 (50%)*
Piperacillin / Tazobactam	1 (10%)	5 (55.56%)	4 (80%)	2 (50%)	1 (50%)	0 (0%)	0 (0%)
Tobramycin			*1 (100%)*			*0 (0%)*	*1 (100%)*

DISCUSSION

The ability of pathogens to develop resistance towards antimicrobial agents makes treatment options and drug development challenging. Therefore, testing for susceptibility to drugs can help prevent prescribing unnecessary and ineffective agents. Out of the positive culture samples, *Pseudomonas* was the most common pathogen isolate followed by *Escherichia coli*. In contrast, another study of sputum culture isolates yielded *Klebsiella pneumonia* in majority.[11] In the current study, *Klebsiella pneumonia* made up only 8% of the total pathogens isolated. The isolates of this species showed highest sensitivity towards Imipenem, Piperacillin / Tazobactam, Gentamicin and Amikacin. These patterns of resistance were very similar to a study done in 2017 in India.[7]

Another study done in Pakistan on resistance patterns of *Pseudomonas* species isolated from burn patients demonstrated resistance towards Gentamicin(92.85%) and Ceftazidime(71%).[8] These results are different from current findings where only 30%

isolates showed resistance to Ceftazidime and only 16.67% were resistant to Gentamicin. These variations in susceptibility patterns are concerning.

A five-year retrospective study done in Cambodia on *Escherichia coli* showed high resistance to Ampicillin(96%) and Cotrimoxazole(86%).[9] These patterns are somewhat similar to current findings where 100% resistance to Ampicillin was observed and 66.67% to Cotrimoxazole. Maximum isolates were highly susceptible to Amikacin and Carbapenems; these findings are in accordance with another study which also showed high susceptibility to these antimicrobial agents.[10]

Staphylococcus aureus was the most common gram positive bacteria isolated from the cultures. The microbe was completely susceptible to Vancomycin and some isolates were resistant to Amikacin. A high number of the isolates were resistant to Cefoxitin and Cephalexin. These findings are consistent with another study from India but decreased resistance to Cephalexin and Cefoxitin was observed compared to the current study.[11]

In our study, *Acinetobacter* species were highly resistant to Amikacin and Ciprofloxacin and moderately resistant to Piperacillin / Tazobactam and high susceptible to Colistin. These results are similar to another study where susceptibility to Piperacillin / Tazobactam and Ciprofloxacin was similar to current results but susceptibility to Amikacin was seen in a few isolates and some isolates were resistant to Colistin.[12] Resistance to Colistin is alarming because several studies on *Acinetobacter* have shown high susceptibility to Colistin.[13,14]

CONCLUSION

Varying patterns of resistance of microbial pathogens were elicited towards antimicrobial agents of routine use; marked resistance was seen towards third generation Cephalosporins and extended spectrum Penicillins. These finding are concerning because they show a trend of increasing resistance which affects treatment decisions and outcome of the disease.

RECOMMENDATIONS

Regular surveillance of changing etiology of infections and their susceptibility patterns is necessary. The pace of resistance can be decreased by taking significant measures such as responsibly prescribing antibiotics based their susceptibility patterns. Antibiograms should be prepared and presented to clinicians regularly to assist in therapy decisions. A central database of antibiograms from different regions will be helpful in formulating treatment guidelines for different infections.

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