RESEARCH PAPER

Bacteriological Profile and Antibiogram of Respiratory Tract Infections in a Tertiary Care Hospital, Bangladesh

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Abstract

Background: Respiratory tract infections (RTIs) are significant health concern for mortality and morbidity in many developing countries. Proper identification of causative pathogens and their antibiotic susceptibility testing is needed to select appropriate antibiotic therapy for management of the patient suffering from RTI. The study was aimed to determine the spectrum of bacterial pathogen causing respiratory tract infections with their antibiogram in Dhaka Medical College hospital (DMCH), Dhaka, Bangladesh.

Methods: This observational study was conducted from October 2018 to March 2019 in DMCH. Respiratory tract specimens (sputum, tracheal aspirate and throat swab) sent to the Microbiology laboratory for culture and sensitivity test were included in this study. Data regarding information of the patients, isolated organisms and sensitivity reports were collected from the records of the Microbiology laboratory.

Results: Out of 580 processed specimens, 64.66% yielded significant growth of organisms of which 88.80% were gram negative and 11.20% were gram positive bacteria. Pseudomonas spp was the most commonly (31.47%) isolated organism followed by Klebsiella spp (23.47%), Escherichia coli (15.20%) and Staphylococcus aureus (8.53%). Gram negative bacteria were mostly resistant to amoxicillin followed by fluoroquinolones, cotrimoxazole, cephalosporins whereas colistin, carbapenems and piperacillin/tazobactum were the most sensitive antibiotics against them. Among gram negative bacteria, 31.23% were extended spectrum beta lactamase (ESBL) producing organisms and Klebsiella spp were the most commonly isolated ESBL producers. Majority of gram positive bacteria were resistant to fluoroquinolones and co-trimoxazole but all Staphylococcus aureus were susceptible to vancomycin and linezolid followed by teicoplanin (84%) and 37.5% of them were Methicillin resistant (MRSA).

Conclusion: Gram negative bacteria were predominant where *Pseudomonas* spp and *Klebsiella* spp were most commonly isolated organisms. Most of the bacteria showed high resistance to commonly used antibiotics and this antimicrobial resistance is a matter of concern for the treatment of respiratory tract infections.

Keywords: Antibiotic susceptibility, ESBL RTI, Bangladesh

Introduction

Respiratory tract infections (RTIs) are among the most common infectious diseases which are considered as major public health problems and leading cause of morbidity and mortality in developing countries. ^{1,2} RTI is a spectrum of infections, each with a different

epidemiology, clinical presentation, pathogenesis and prognosis. The etiology, clinical features of respiratory diseases vary with age, gender, season, the type of population at risk and various other factors.³ The etiological agents of respiratory tract infections cannot be determined clinically and differ from area to area as well as their antibiotics susceptibility.⁴

The common bacterial causes of respiratory infections include *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter* spp and *Haemophilus influenzae*.^{4,5} The responsible pathogens are identified

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in about half of the patients and physicians usually rely on clinical signs and symptoms of the patient to diagnose respiratory tract infections. ^{6,7} Recommendations of initial therapy are based on the severity of illness, the probabilities of the pathogens in specific geographical areas, resistance patterns of the most commonly implicated etiological agents and co-morbidities. ⁸

The dramatic rise in the antimicrobial resistance among the respiratory pathogens is a matter of potential concern worldwide.9 Excessive and inappropriate use of antibiotics is considered as a major cause of antibiotic resistance in developing country. The increased use of over-the-counter antibiotics not only produces resistance at the individual level but can also threaten the whole community. 10 Over the last few decades, multidrug-resistant bacterial strains such as Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Methicillin resistant Staphylococcus aureus (MRSA) are increasingly associated with infections under hospital settings. 11,12 Regular monitoring of antimicrobial susceptibility profile, cautious and judicious use of antibiotics could be effective tools to prevent the spread of antimicrobial resistance. 13,14

As the antibiotic resistance pattern of bacteria is changing and no recent data on causative pathogens of RTIs and their antibiotic susceptibility pattern is available in Dhaka Medical College, the present study was designed to evaluate the etiological agents of respiratory tract infections with their antibiogram in this tertiary care hospital.

Materials and Methods

This observational study was conducted in the Department of Microbiology, Dhaka Medical College, Dhaka, Bangladesh from October 2018 to March 2019. Respiratory tract specimens (sputum, tracheal aspirate and throat swab) sent from the inpatient and outpatient departments of Dhaka Medical College Hospital to the microbiology laboratory for culture and sensitivity test regardless of age, sex and antibiotic intake were included in this study. Data regarding the information of patients, referring departments, types of specimen and sensitivity reports were collected from the records of the laboratory.

Microbiological methods:

Culture of sputum, tracheal aspirate and throat swab: In the microbiology laboratory, each sample was inoculated on Blood agar, Chocolate agar and MacConkey agar media. The blood agar and MacConkey agar plates were incubated aerobically at 37°C for 48 hours while the chocolate agar plates were incubated in a carbon dioxide enriched environment using Candle jar at 37°C for 48 hours.

Isolation and identification of bacteria: The inoculated plates were examined for bacterial growth and organisms were identified by colony morphology, hemolytic criteria, pigment production, Gram stain and different biochemical tests like catalase test, coagulase test, oxidase test, and reaction in Triple Sugar Iron (TSI) agar, Motility Indole Urea (MIU) agar and Simmon's citrate agar media. 15

For evaluation purpose Gram stain of sputum was done and correlated with culture result to differentiate between true infection and commensal or contamination. (Gram stain with <10 squamous epithelial cells and >25 leucocytes/ low power field were considered significant).

Antimicrobial susceptibility testing: Antimicrobial susceptibility pattern of isolated organisms were done following Kirby-Bauer disc diffusion method 17 using commercially available antibiotic discs (Oxoid, UK) such as, amikacin (30 μ g), amoxyclav (20 μ g amoxycillin/10 μ g clavulanic acid), cefoxitin (30 μ g), ceftazidime (30 μ g), ceftriaxone (30 μ g), clindamycin (2 μ g), ciprofloxacin (5 μ g), colistin (10 μ g), doxycycline (5 μ g), gentamicin (10 μ g), imipenem (10 μ g), levofloxacin (5 μ g), linezolid (30 μ g), meropenem (10 μ g), piperacillin/tazobactum (100/10 μ g), teicoplanin (30 μ g), vancomycin (30 μ g). Zone of inhibition was measured and interpreted according to the CLSI guideline. 18

Detection of Methicillin resistant Staphylococcus aureus (MRSA): Methicillin resistance of Staphylococcus aureus was detected using cefoxitin disc (30 µg) according to CLSI guidelines.¹⁸

Detection of Extended spectrum beta lactamase (ESBL): ESBL production in gram negative bacteria was detected by double disc synergy test.¹⁸

Data management: Collected data were classified according to characteristics and categorical data were given as counts and percentages. 'Microsoft Excel' 2016 software were used for analysis.

Results

Total 580 respiratory tract specimens were processed among which sputum was 335, tracheal aspirate was

202 and throat swab was 43. Significant growth was found in 64.66% of total specimens; tracheal aspirate yielded the highest proportion of microbial growth (77.23%) followed by sputum (58.51%) and throat swab (53.49%) (Table-I).

Among 375 culture positive specimens 333 (88.80%) yielded growth of gram negative bacteria and 42 (11.20%) had growth of gram positive bacteria. In sputum, 170 (86.73%) were gram negative and 26 (13.27%) were gram positive bacteria; in tracheal aspirate, 150 (96.15%) had growth of gram negative and 6 (3.85%) were gram positive bacteria and in throat swab, isolated gram negative and gram positive bacteria were 13 (56.52%) and 10 (43.48%) respectively (Figure-1).

Out of 375 isolated organisms *Pseudomonas* spp was the most predominant (31.47%). *Klebsiella* spp was isolated from 23.47% specimens, 15.20% specimens yielded growth *of E. coli* and *Acinetobacter* spp was isolated from 6.67% samples. *Staphylococcus aureus* was the most frequently isolated (8.53%) gram positive

bacteria followed by *Streptococcus pneumoniae* (Table-II).

Among the gram negative bacteria, 31.23% (104/333) isolates were ESBL producers. *Klebsiella* spp were the most commonly isolated ESBL producers, 30.77% of which produced ESBLs. Among *Escherichia coli* 17.31% were ESBL producers; 16.35% of *Acinetobacter* spp, 13.46% *Proteus* spp and 9.61% *Enterobacter* spp were ESBL producing organisms Figure-2).

Most of the gram negative bacteria exhibited higher sensitivity to carbapenems. Colistin was found most effective antibiotic in vitro against all gram negative except *Proteus* spp Piperacillin/tazobactum showed greater activity against *Enterobacter*, *Citrobacter* spp, *Proteus* and *E. coli* (Table-III).

Staphylococcus aureus showed 100% susceptibility to vancomycin and linezolid followed by high susceptibility to teicoplanin. Among 32 isolated Staphylococcus aureus, 12 (37.5%) were MRSA strain (Table-IV).

Table I: Growth of bacteria in different specimens

Type of specimen	Growth n(%)	No growth n(%)	Total n(%)
Sputum	196 (58.51)	139 (41.49)	335 (100)
Tracheal aspirate	156 (77.23)	46 (22.77)	202 (100)
Throat swab	23 (53.49)	20 (46.51)	43 (100)
Total	375 (64.66)	205 (35.34)	580 (100)

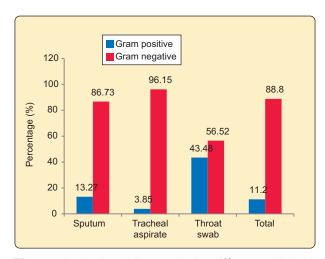


Figure-1: Isolated bacteria in different clinical specimens.

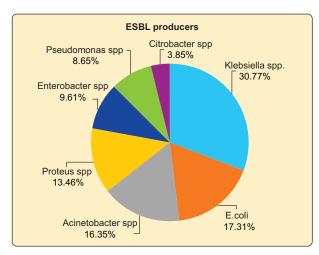


Figure-2: Distribution of ESBL producing gram negative organisms.

Table II: Pattern of isolated bacteria from sputum, tracheal aspirate and throat swab.

Organism	Sputum	Tracheal aspirate	Throat swab	Total		
	Isolates n (%)	Isolates n (%)	Isolates n (%)	Isolates n (%)		
Pseudomonas spp	49 (25.00)	66 (42.31)	3 (13.04)	118 (31.47)		
Klebsiella spp	63 (32.14)	18 (11.54)	7 (30.43)	88 (23.47)		
E. coli	35 (17.86)	19 (12.18)	3 (13.04)	57 (15.20)		
Staph. aureus	21 (10.71)	6 (3.85)	5 (21.74)	32 (8.53)		
Acinetobacter spp	3 (1.53)	22 (14.10)	0 (0.00)	25 (6.67)		
Proteus spp	0 (0.00)	18 (11.54)	0 (0.00)	18 (4.80)		
Enterobacter spp	9 (4.59)	4 (2.56)	0 (0.00)	13 (3.47)		
Citrobacter spp	10 (5.10)	3 (1.92)	0 (0.00)	13 (3.47)		
S. pneumoniae	5 (2.55)	0 (0.00)	5 (21.74)	10 (2.67)		
H. influenzae	1(0.51)	0(0.00)	0(0.00)	1(0.27)		

Table III: Antibiotic sensitivity pattern of isolated gram negative bacteria.

Organism	Sensitivity (%)												
	Ak	Aml	Amc	Caz	Ctr	Cip	Cot	L	G	Imp	Mem	TZP	CT
Pseudomonas	30	-	8	54	13	21	19	13	31	64	68	61	97
Klebsiella	49	-	8	14	16	19	31	48	32	85	84	65	92
E. coli	46	7	9	18	21	23	19	39	40	91	82	70	95
Acinetobacter	12	-	4	0	8	8	24	12	8	40	36	32	100
Proteus	28	3	11	17	17	22	39	33	22	89	78	72	5
Enterobacter	54	8	8	23	15	15	38	31	31	92	85	77	92
Citrobacter	54	14	23	23	23	31	54	38	62	85	85	77	100
H. influenzae	100	-	-	100	100	100	-	-	-	100	100		

Ak-amikacin, Aml-amoxycillin, Amc-amoxyclav, Caz-ceftazidime, Ctr-ceftriaxone, Cip-ciprofloxacin, Cot-Cotrimoxazole, L-levofloxacin, G-gentamicin, Imp-imipenem, Mem-meropenem, TZP-piperacillin/tazobactum, CT-colistin.

Table IV: Antibiotic sensitivity pattern of gram positive bacteria.

Organism	Sensitivity (%)												
	Ak	Aml	Ctr	Cip	Cot	D	G	L	Lzd	CX	CD	Tec	Van
Staph. aureus	44	-	-	22	28	38	41	25	100	38	75	84	100
S.pneumoniae	50	80	100	50	50	50	70	60	-	-	-	-	-

Ak-amikacin, Aml-amoxicillin, Ctr-ceftriaxone, Cip-ciprofloxacin, Cot-Co-trimoxazole, D-doxycycline, G-gentamicin, L-levofloxacin, Lzd-linezolid, CX-cefoxitin, CD-clindamycin, Tec-teicoplanin, Van-vancomycin.

Discussion

Respiratory tract infections are among the most common infectious diseases causing significant morbidity and mortality. An expanded variety of emerging pathogens provides challenges for the microbiology laboratory. ¹⁹ In recent years, there has been substantial rise in antibiotic resistance among respiratory pathogens. The present study is an attempt to provide an insight on the prevalence and the antibiogram pattern of the respiratory pathogens which were isolated in a tertiary care hospital.

Among the specimens, 375 (64.66%) yielded significant growth of different bacteria which is consistent with

other studies.^{20,21} Similar to other study done in Assam, India; in this study 58.51%, 77.23% and 53.49% of the pathogens were recovered from sputum, tracheal aspirate and throat swab respectively.²²

Most of the isolated organisms (88.80%) from respiratory tract specimens were gram negative bacilli. In contrast, gram positive bacteria were reported to be most prevalent in other study. Higher proportion of gram negative bacteria isolated in the present study might be due to the fact that most of the samples were received from admitted patients of DMCH and many of them might have hospital acquired infection as gram negative bacteria is usually prevalent in hospital.

Pseudomonas spp. was the most (31.47%) predominant isolated organism among gram negative bacteria followed by Klebsiella spp, E.coli, Acinetobacter and Proteus spp. In accordance with the present study, similar findings were observed in Nepal.²⁴ In contrast, lower isolation rate of Pseudomonas spp was reported in study in India.²⁵ Higher isolation rate of Pseudomonas spp might be due to the fact that majority of the respiratory tract specimens were sent from inpatient department and intensive care unit (ICU) of the hospital. Pseudomonas spp is a ubiquitous organism that it could affect individual with immunocompromised situation and responsible for nosocomial infection.²⁶ It has not only metabolic versatility and remarkable ability to adaptation and colonization in wide variety of ecologic environments (water, soil, animals), but also notability for its intrinsic ability to resistance to wide variety of antimicrobial agents.²⁷

In consistent with other studies^{28,29}, *Klebsiella* spp (23.47%) was the second common pathogen isolated in the present study. But in contrast, *Klebsiella* spp was the most predominant bacteria isolated from lower respiratory tract infections in India.⁵ This might be due to the fact that the spread of respiratory tract infections varies between populations and countries, depending on differences in geography, climate and socioeconomic condition.

In our study Staphylococcus aureus was the most frequently isolated gram positive bacteria from respiratory samples which was similar to other studies. 20,30 About 37.5% of the isolated Staphylococcus aureus were Methicillin resistant Staphylococcus aureus (MRSA). In Bangladesh, the rate of MRSA infection ranges from 32% to 63% in different studies³¹ which is in accordance to the isolation rate in this study. MRSA is a multidrug resistant bacterium which is resistant to methicillin, other penicillins and most cephalosporins, \(\beta\)-lactam/ β -lactamase inhibitor combinations and carbapenems. 18 Only 2.67% Streptococcus pneumoniae was isolated. This is comparable to a study done in India where 3.19% of Streptococcus pneumoniae have been reported.²⁵ It has been estimated that, even in patients with S. pneumoniae pneumonia, the usual laboratory methods cannot detect the pathogen in 45-50% of cases even when large numbers of organisms have been noted on gram stain.32

The antimicrobial resistance among the respiratory pathogens is a major barrier interfering an effective treatment.²⁵ In the present study, gram negative isolates showed higher resistance patterns towards amoxicillin followed by fluoroquinolones, co-trimoxazole, amoxicillinclavulinic acid and third generation cephalosporins. This pattern of resistance has been shown by several studies. 25,33 Inappropriate and widespread use of this antibiotics has been suggested as one of the reasons of this resistance. The emergence of fluoroquinolones resistance among RTIs has now been documented in many countries .34,35 Fluoroquinolones are important antibiotic used widely in Bangladesh to treat various types of infection. Nowadays, fluoroquinolones are substituted by the 3rd generation cephalosporins, which are frequently used by clinicians. Most of the gram negative bacilli showed good sensitivity to amikacin in the present study which is in accordance to other study done in India.²⁰ This may be due to selective use of amikacin in our setup because of higher adverse effects of this injectable antibiotic.

Carbapenems are very effective antibiotics and are widely used against gram negative bacilli. Sensitivity to carbapenems were 64% to 92% against gram negative bacilli in this study which is consistent with other study. Carbapenems resistance is increasing day by day and it is a matter of great concern in management of infection. Colistin and piperacillin/tazobactum showed better sensitivity in vitro. Most of the gram negative bacilli were sensitive to colistin except *Proteus* spp. *Proteus* spp is intrinsically resistant to colistin by modification of the lipopolysaccharide (LPS) via cationic substitution. Colistin and piperacillin/tazobactum are usually considered as reserve drug and are being used for those who are resistant to most other antibiotics.

All gram positive bacteria were sensitive to vancomycin and linezolid. All the *Staphylococcus aureus* were sensitive to vancomycin and linezolid followed by teicoplanin (84%) and had reduced sensitivity to ciprofloxacin (22%), levofloxacin (25%) and cotrimoxazole (28%) which was similar to the sensitivity pattern of *Staphylococcus aureus* in a study in Bangladesh.³⁷

The emergence of multidrug resistant strains possesses a major threat to the patients globally. Among the gram negative isolates, various mechanisms of drug resistance have been attributed, among which production of beta-lactamases is a leading cause of resistance.²⁵ Besides, not all the bacteria causing RTIs

are identified by conventional culture and sensitivity method and antimicrobial resistance containment program including antimicrobial stewardship is not very effective in countries like Bangladesh. As a result, multidrug resistant strains of microorganism are increasing. In our study 31.23% bacterial isolates were ESBL producers which is in accordance with the study done in India.²⁰ In Bangladesh in a study the rate of ESBL producing bacteria were 16.07% which is lower than the present findings. 38 Klebsiella spp and E. coli were the most commonly isolated ESBL producing organisms in this study. ESBL producing bacteria limit the therapeutic options for treatment; therefore, strategies for laboratory detection of ESBL producing bacteria as well as antimicrobial susceptibility testing are important.39

Conclusion

Gram negative bacilli were the predominant isolates of respiratory tract infection with *Pseudomonas* spp as the most common isolate. Occurrence of multidrug resistance, MRSA and ESBL producing bacteria should be considered as a cause of concern. Appropriate and evidence based antibiotic treatment policy should be practiced. However, a combined approach of antimicrobial stewardship, continuous surveillance of microbial etiology of RTI with their resistance pattern and good infection control practices will help to reduce the burden of multidrug resistance and thereby enabling better patient management.

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Conflict of Interest: We do not have any potential conflicts of interest.

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Ethical approval: The study was conducted with the prior permission from the Department of Microbiology of Dhaka Medical Collage. Anonymity of the patients and confidentiality of information was maintained strictly.

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