

Recent trend of bacterial aetiology of respiratory tract infections with special reference to *Escherichia Coli*

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Abstract

Background and Objective: Respiratory tract infections (RTIs) are among the most common infectious diseases affecting humans worldwide. Pathogenic isolates of *E.coli* have relatively high potentials for developing resistance. Therefore, the treatment of *E. coli* infections is increasingly becoming difficult.

Objectives: To isolate and identify the bacterial pathogens in sputum samples causing Respiratory tract infections and to know the prevalence and sensitivity pattern of *E.coli* in sputum samples.

Materials and Methods: Sputum samples were collected from 1st January 2014 to 31st December 2014 at VIMS Hospital, Ballari from the patients of suspected RTIs and processed for Gram's stain and biochemical reactions. Antibiotic susceptibility test was done by Kirby-Bauer disc diffusion method and Extended Spectrum β -Lactamases (ESBL) detection performed as per CLSI guidelines.

Results: A total of 1264 sputum samples were processed, out of which 756(59.8%) yielded growth. The bacteria isolated from the samples included *Klebsiella spp* 261(34.5%), *Staphylococcus aureus* 142(18.8%), *Streptococcus pneumonia* 121(16%), *Streptococcus pyogenes* 96(12.6%), *Pseudomonas* 85(11.2%), *Escherichia coli* 21(2.8%) *Citrobacter spp* 18(2.3%) and *Enterococci* 12(1.8%). *E.coli* showed significant resistance to Ampicillin (90.5%), Ciprofloxacin (81%), Amoxiclav (76.2%), Cefotaxime (71.4%) and Cotrimoxazole (66.7%). It was 100% sensitive to Imepenem followed by Amikacin (61.9%) and Gentamicin (57.1%). ESBL production was seen in 76.2% of *E.coli* isolated.

Conclusion: There is a need to develop antibiotic policy and this will provide valuable insight on resistance trends and encourage the prudent use of antibiotics, which is a major factor in controlling the emergence and spread of resistant strains.

Keywords: Respiratory Tract Infections, Sputum, *Escherichia coli*, Antimicrobial Resistance, Extended Spectrum β -Lactamases (ESBL).

Introduction

Respiratory tract infections (RTIs) are one of the most common infectious diseases affecting humans worldwide. They are an important cause of morbidity and mortality in many developing countries.⁽¹⁾ It is a global problem accounting for over 50 million deaths of each year and occurs in both community and health care settings.⁽²⁾ The microorganisms larger than 10 μ m usually are trapped by the hair and cilia lining the nasal cavity. Coughing and sneezing reflexes clear the respiratory system of microorganisms by expelling them forcefully from the lungs through the mouth and nose respectively.⁽³⁾

Most of RTIs are limited to the upper respiratory tract and only 5% involve the lower respiratory tract. Upper Respiratory Tract infections (URTIs) involve the nasal passages, pharynx, tonsils and epiglottis whereas Lower respiratory tract infections (LRTIs) involve the bronchi and alveoli which cause two serious conditions—acute bronchitis and pneumonia.⁽⁴⁾

Escherichia coli is associated with infections of the gastrointestinal tract, urogenital tract, and peritoneum and occasionally with infections at distant loci after bacteremia.⁽⁵⁾ It is one of the most important members of the Enterobacteriaceae. Strains of *E.coli* are usually present as aerobic commensals in the healthy human intestine.⁽⁶⁾ It is rarely associated with pulmonary

infections. *E. coli* causes extra intestinal infections due to the presence of several virulence factors which help in the survival of *E. coli* under adverse conditions which are present at those sites.⁽⁷⁾

Antimicrobial resistance in *E.coli* has been increasing worldwide. Therefore, the treatment of *E.coli* infections is increasingly becoming difficult and is a growing concern in both developed and developing countries. Extended spectrum β -lactamase (ESBL) producing organisms are a major problem in the treatment of infections.^(8,9)

Hence a retrospective study is undertaken to know the bacterial aetiology of respiratory tract infections with special reference to *Escherichia coli*.

Objectives of the study

1. To isolate and identify the bacterial pathogens in sputum samples causing Respiratory tract infections.
2. To know the prevalence and sensitivity pattern of *E.coli* in sputum samples.

Materials and Methods

Study includes both in and out patients with history of cough and breathlessness seeking medical attention at VIMS Medical College Hospital, Ballari. The study was

carried out at VIMS Medical College Hospital, Ballari, from 1st January 2014 to 31st December 2014.

Sputum samples were collected from patients with history of cough and breathlessness. Early morning samples were collected before the antibiotic therapy from the symptomatic patients. All the samples were processed for Gram's stain and cultured onto blood agar and Mac conkey agar within two hours of collection. The agar plates were incubated overnight at 37^o C aerobically and examined for the presence of any growth after overnight incubation.

The isolates were identified by colony morphology, Gram's stain and biochemical reactions and antibiotic susceptibility tests performed by CLSI recommended by Kirby-Bauer disc diffusion method.

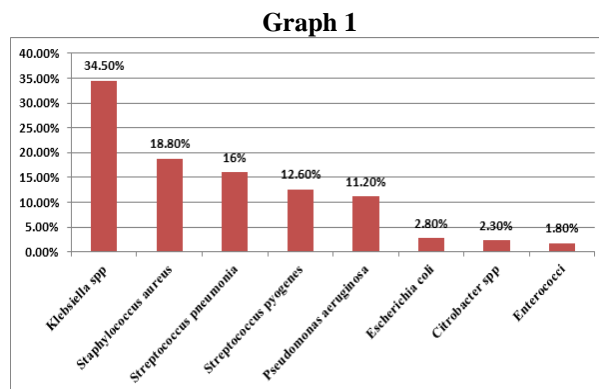
The kirby bauer disk diffusion method⁽⁹⁾: Mueller Hinton agar (Hi Media, Mumbai) was prepared from a dehydrated base according to the manufacturer's instructions. The inoculum was prepared by emulsifying 3-5 morphologically similar colonies in peptone broth and incubating them at 37°C until the turbidity was matched to a 0.5 Mc Farland's Turbidity standard. *E coli* ATCC 25922 strain was used to prepare the control.

A sterile cotton swab was dipped into the inoculum and rotated several times against the wall of the test tube above the fluid level to remove the excess inoculum. The dried surface of a Mueller Hinton plate was then inoculated with the swab as a lawn culture. Once the surface was dried, the antibiotic disks (from Hi Media, Mumbai) were placed on the surface by evenly spacing them. The distance between any two disks was 24mm from centre to centre. The plates was incubated overnight at 37°C, the inner diameter of the zone of inhibition was measured by using a millimetre scale around each antimicrobial disk, on the under surface of the plate. The zone size around each antimicrobial disk was interpreted as sensitive or resistant according to the CLSI guidelines.⁽¹⁰⁾

ESBL Producers were detected by combination disk method using Cefazidime (30 µg) and Cefazidime + Clavulanic Acid (20 µg + 10 µg) were placed at the distance of 20mm from centre to centre. Plates were incubated at 37°C overnight. A > 5mm increase in zone diameter for the antimicrobial tested in combination with Clavulanic Acid versus its zone when tested alone confirmed ESBL production.⁽¹¹⁾

Results

A total of 1264 sputum samples were collected during the study period, out of which 756(59.8%) yielded growth. Commensals were isolated in 508(40.2%) cases. The bacteria isolated from the samples included *Klebsiella spp* 261(34.5%), *Staphylococcus aureus* 142(18.8%), *Streptococcus pneumonia* 121(16%), *Streptococcus pyogenes* 96(12.6%), *Pseudomonas aeruginosa* 85(11.2%), *Escherichia coli* 21(2.8%), *Citrobacter spp* 18(2.3%) and *Enterococci* 12(1.8%). (Graph 1)



Of the 21 *E.coli* isolated, antibiotic susceptibility testing was done and looked for ESBL production. The organism was more common in males 15(71.4%) compared to females 6(28.6%). (Table 1) *E.coli* showed significant resistance to Ampicillin (90.5%), Ciprofloxacin (81%), Amoxiclav (76.2%), Cefotaxime (71.4%) and Cotrimoxazole (66.7%). It was 100% sensitive to Imepenem followed by Amikacin (61.9%) and Gentamicin (57.1%). (Table 2) ESBL production was seen in 16(76.2%) of *E.coli* isolated. (Graph 2)

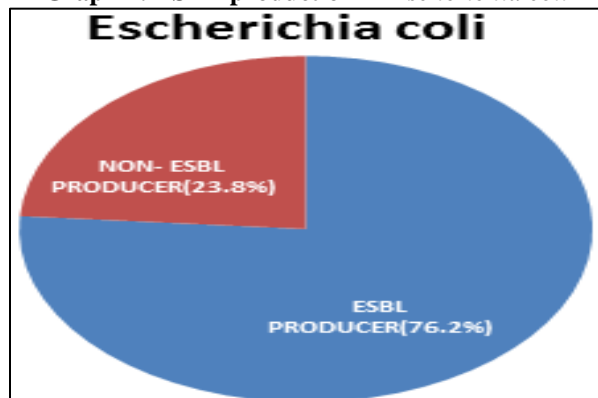
Table 1: Age and gender wise distribution of *Escherichia coli* in sputum

Age group (years)	Male	Female	Total
Upto 10	0	0	0
11-20	0	0	0
21-30	1	1	2
31-40	4	2	6
41-50	3	1	4
51-60	4	1	5
61-70	3	1	4
Total	15(71.4%)	6(28.6%)	21(100%)

Table 2: Antibiotic susceptibility pattern of *Escherichia coli*

	Amp	AMC	Cip	Ctx	Gen	Ak	Cot	Imp
S(%)	2(9.5)	5(23.8)	4(19.0)	6(28.6)	12(57.1)	13(61.9)	7(33.3)	21(100)
R(%)	19(90.5)	16(76.2)	17(81.0)	15(71.4)	9(42.9)	8(38.1)	14(66.7)	0(0)

Amp- Ampicillin, AMC- Amoxycrav, Cip- Ciprofloxacin, Ctx- Cefotaxime, Gen- Gentaicin, Ak- Amikacin, Cot- Cotrimoxazole, Imp- Imepenem

Graph 2: ESBL production in *Escherichia coli*

Discussion

Out of 1264 samples analysed 756 samples yielded growth. Among the various bacteria isolated, *Klebsiella* spp. (34.5%) was the most common isolate followed by *Staphylococcus aureus* (18.8%), *Streptococcus pneumoniae* (16%), *Streptococcus pyogenes* (12.6%), *Pseudomonas* (11.2%), *Escherichia coli* (2.8%) *Citrobacter* spp (2.3%) and *Enterococci* (1.8%) respectively.

Of the 21 *E.coli* isolated, the organism was more common in males 15(71.4%) compared to females 6(28.6%). Significant resistance was seen to Ampicillin (90.5%), Ciprofloxacin (81%), Amoxiclav (76.2%), Cefotaxime (71.4%) and Cotrimoxazole (66.7%). It was 100% sensitive to Imepenem followed by Amikacin (61.9%) and Gentamicin (57.1%). ESBL production was seen in 76.2% of *E.coli*.

In a study done by Asati Rakesh Kumar, 512 sputum samples were collected and processed.

The most common pathogens were *K.pneumoniae* (39.5 %) followed by *Pseudomonas* (25 %), *E. coli* (11.5 %), *Staphylococci* (11.5%) and others (3.8%). This was similar to our study where *Klebsiella* spp was the predominant organism isolated.⁽¹²⁾

C. Manikandan and A. Amsath processed 360 specimens, out of which 337(93.6%) species of various bacteria were isolated. The prevalence of bacteria spp. isolated were as follows *Streptococcus pneumoniae* (36%), *Klebsiella pneumoniae* (28.4%), *Staphylococcus aureus* (24%), *Pseudomonas aeruginosa* (11%) and *Escherichia coli* (0.6%).⁽⁴⁾ In this study *Streptococcus pneumoniae*(36%) was the predominant organism unlike our study.

In a study done by Asima Banu et al, 11 sputum isolates were *E.coli*, out of 538 samples with significant resistance to Ampicillin (95.6%), Cotrimoxazole (69.5%). Ciprofloxacin (65.2%), Gentamicin (52.2%) and 100% sensitive to Imepenem followed by Cefotaxime (69.6%) Amikacin (60.9%).⁽⁸⁾

A study conducted by Manu Chaudhary and Anurag Payasi showed that, out of 84 *E.coli* in the sputum 28 (33.3%) were ESBL producers.⁽¹³⁾

E.coli resistance has increased worldwide over the past decade. In this study, the overall resistance of *E. coli* to antimicrobial agents was high. This may be due to increasing irrational use of antibiotics, self-medication because of over-the-counter availability of antibiotics, patients not adhering for full course of antibiotics dosage, consumption of food from animals that have received antibiotics and transmission of resistant isolates between people in the community.

Conclusion

This study was done to recognize the most common bacterial agent of RTIs in our hospital and also the prevalence of *E.coli* in the sputum samples. There was an increasing trend of drug resistance among *E.coli* causing Respiratory Infections with significant Extended Spectrum B – Lactamases (ESBL) producers. Thus, there is a need to develop antibiotic policy in every hospital to reduce the occurrence of resistant strains and also their spread in the community. This in turn will help in the better outcome of patients suffering from life threatening infections.

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