International Research Journal of Microbiology (IRJM) (ISSN: 2141-5463) Vol. 3(2) pp. 045-049, February 2012
Available online http://www.interesjournals.org/IRJM

Full Length Research Paper

Bacterial Habitat of Lower Respiratory Tract with Antibiotic Resistance in Sanitary Workers

Sankar Ramachandran*, Elavarasan Subramani, Koel Chaudhury and Mahitosh Mandal

School of Medical Science and Technology, Indian Institute of Technology Kharagpur, Kharagpur – 721302, West Bengal, India.

Accepted 06 February, 2012

The sanitary workers are more prone to increased risk for several airway symptoms, chronic bronchitis and pneumonitis. Lower respiratory tract infections (LRTI) are more prevalent and one of the major causes of death in developing countries. Respiratory tract contains a number of distinct ecosystems, each with its unique microbial flora. The present study was to determine the microbial profile and its antibiotic susceptibility in lower respiratory tract (LRT) of sanitary workers. Seventy nine respiratory samples from LRT of sanitary workers were analyzed. Differential and selective media were used for the identification of bacterial isolates of LRT. Antibiotic susceptibility test has been done to identify the sensitivity of predominant bacterial isolates. Among the isolated organisms, *Pseudomonas aeruginosa (P. aeruginosa), Staphylococcu aureus (S. aureus), Streptococcus pneumonia (S. pneumoniae), Beta heamolytic Streptococci* and *Klebsiella* were found to be more prevalent. Antibiotic susceptibility pattern of both Gram positive and Gram negative bacterial isolates of LRTI includes *S. aureus* and *P. aeruginosa* shows significantly higher resistance to the antibiotics. Clinical and bacteriological efficacy and its broad spectrum antibiotics of LRTI may be of assistance to find new era of prevention and treatment.

Keywords: LRTI, sanitary workers, antibiotics, resistance, susceptibility.

INTRODUCTION

Over decades, sanitary workers have remained almost unchanged in their working surroundings and socioeconomic status. They are exposed to certain health problems including cardiovascular degeneration, musculoskeletal disorders, leptospirosis, hepatitis, skin problems, and respiratory problems (Melbostadt et al., 1994). It is evident that sewer workers have 53.8% developed dyspnoea, cough, sore throat, skin irritability, airway symptoms and chest tightness (Melbostadt et al., 1994). Studies on respiratory function of sewage workers revealed abnormal airway passage, chronic respiratory infection by virtue of their occupation (Melbostadt et al., 1994; Thorn J et al., 2002). Exposures of various occupational deleterious agents lead to the development of decreased lung function and increased risk for asthma and chronic bronchitis (Schwartz DA et al., 1995).

Respiratory tract infection (RTI) is one of the leading causes of morbidity and mortality among sewage workers (Zuskin E et al., 1993). RTI is responsible for five million deaths per year, out of this 10-15% are due to lower RTI (LRTI) throughout the world (Lusuardi M et al., 2003). Both upper and lower respiratory tracts are protected from inhaled particles naturally, by many mechanisms. In respect to this, certain micro organisms are considered to be aetiologic agent of diseases, evade the host immune system by multiplying within the host cells. The specific type of respiratory infection is caused by a variety of factors, including age, season, anatomic features of the airway, type of population at risk and others (Ejlertsen T et al., 1992; Zuskin E et al., 1990). LRTI is usually the result of either bacterial or viral invasion of lung parenchyma. For the most part, infection in the lower respiratory tract occurs when host defence mechanisms

*Corresponding Author E-mail: Shankarsha24@gmail.com; Phone: 91+8086541984

break down due to chronic inflammation associated with an irritant such as cigarette smoke or due to immune deficiency. LRTI including community acquired pneumonia (CAP), acute exacerbation of chronic bronchitis (AECB) and chronic obstructive pulmonary diseases (COPD) are the most prevalent and fatal infectious diseases (Lusuardi M et al., 2003; Sethi S, 2001a).

In general agreement that the bacterial species most commonly isolated from sputum and lower respiratory tract during respiratory infections are *Heamophilus influenza (H. influenza), Streptococcus pneunoniae (S. pneumoniae), Pseudomonas aeruginosa (P. aeruginosa)* and *Moraxella catarrhalis (M. catarrhalis)* (Jacobs MR et al., 1994). In recent years, the emergence of antibiotic resistance among common respiratory pathogens has drawn attention. It is difficult to decide whether patient characteristics or the risk of antibiotic resistance should influence choice of empiric antibiotic treatment (Craig WA, 2001). There is inadequate information on various lower respiratory tract bacterial pathogens and their resistance patterns among sanitary workers.

Therefore, to better understand perceptions of urban sanitary workers about the need of management of different types and severities of LRTIs, a prospective study was performed to investigate the common bacterial profile and its frequency of drug resistant in lower respiratory tract of urban sanitary workers.

MATERIALS AND METHODS

Study Cohort

Seventy-nine sanitary male workers (age 41.5, range 32-51) working in urban areas and its surroundings of West Bengal, India were enrolled in this prospective study. Respiratory secretions sputum (rarely nasopharyngeal and throat swab) was collected in a sterile wide mouth glass container, homogenized for 10-15 min in vortex mixer after written informed consent obtained. Subjects with pulmonary tuberculosis, acute bacterial infections, therapeutic immune suppression, malignancy and history of smoking and/or alcoholism were excluded from this study.

Bacteriological Analysis

Respiratory secretions were obtained by means of sputum collection from each of the subjects were cultured in both selective and non-selective autoclaved media at 37^{o}C. Next day the colony morphology and number of colonies were noted. Pure, isolated colonies of each type was separately cultured and characterized. Gram and motility nature of the each isolates was determined by observing at 40X in bright field microscope.

Biochemical characterization

Carbohydrate Fermentation test

Microorganisms' ability to ferment specific carbohydrate was determined using Phenol red as indicator. Sterilized carbohydrates (5-10%) include glucose, sucrose, mannose, maltose, fructose, lactose and inositol were added to sterile peptone broth (1% Peptone, 1% Beef extract and 0.5% NaCl). A loopful of test organism was inoculated into 5-10ml individual carbohydrate broth and incubated at 37^{0}C for 18-24 hours.

IMViC Test

IMViC (Indole Methylred Voges-Proskauer Citrate) test was done by inoculating test colony into the test tubes containing tryptone broth and MRVP broth respectively. The changes in color of tubes were observed within 15 minutes by adding respective reagents (Kovac's reagent, MR reagent and VP A & B reagent). Citrate can be used as a sole carbon source by certain microorganisms. A drop of culture was inoculated into the citrate agar slant and incubated at 37^{0}C for 18-24 hours.

Urease Test

Certain microorganism produces urease, the enzyme which degrades urea. Microbial culture was inoculated into the sterilized Urea agar slant and incubated for 18-24 hours at 37^{0}C. Phenol red was incorporated into to the medium as an indicator to find color changes from yellow to red in the presence of urease.

Catalase Test

The catalase producing organisms was determined by applying 3-5% Hydrogen peroxide (Merck, India) over the colonies on the solid media.

Oxidase Test

Bacterial colony from solid media was brought into contact with oxidase disc (Himedia Laboratories Ltd.) to test the presence of enzyme cytochrome oxidase and change in color of disc observed within 60 seconds.

Antibiotic susceptibility test

The sensitivity of each isolates against various antibiotics was determined by disc diffusion test according to Kirby-Bauer method. The zones of inhibition were recorded for

Table 1. Morphological characterization of the bacterial isolates observed under microscope

Microorganisms	Cell morphology	Gram nature	Motility
Pneumococci	Coccus	Gram Positive	Non motile
Betahemolytic streptococci	Coccus	Gram Positive	Non motile
Staphylococcu aureus	Coccus	Gram Positive	Non motile
Klebsiella	Bacillus	Gram Negative	Non motile
Staphylococcu epidermidis	Coccus	Gram Positive	Non motile
Pseudomonas aeruginosa	Bacillus	Gram Negative	Motile
Serratia	Bacillus	Gram Negative	Motile
E.coli	Bacillus	Gram Negative	Motile
Micrococci	Coccus	Gram Positive	Non motile
S.viridians	Coccus	Gram Positive	Non motile

Table 2. Biochemical characterization of different bacterial isolates

Micororganisms	IMViC	Sugar Fermetation	Urease	Catalase	Oxidase
Pneumococci	-	Lac	-	-	-
Beta hemolytic streptococci	-	Mann	-	-	-
Staphylococcu aureus	-++d	-	+	+	+
Klebsiella	--++	F,S,M,I	+	+	-
Staphylococcu epidermidis	-	G,I,S	+	+	-
Pseudomonas aeruginosa	---d	S,M	+	+	-
Serratia	-d++	M,S,G	-	+	-
E.coli	++--	M,S	-	+	-
Micrococci	-	-	-	+	-
S.viridians	-	-	-	-	-

Note: Presence of respective property (+) i.e. showing positive biochemical reaction, absence of respective property (-), d- , G-Glucose, S-Sucrose, M-Maltose, I-Inositol, F-Fructose, Lac-Lactose, Mann-Mannose.

all the plates and the antibacterial results expressed as susceptible, intermediately resistant and resistant using National Committee for Clinical Laboratory Standards criteria. The MICs of antimicrobial drugs, Ampicillin (A), Chloramphenicol (C), Erythromycin (E), Penicillin (P), Tetracyclin (T), Cloxacillin (Cx), Nalidixic acid (NA), Ciprofloaxacin (CIP), Gentamicin (G), Ceftriaxone (CF), Norfloxacin (NX) for antibiotics were determined by microdilution method. Muller Hinton Agar (Himedia Laboratories Ltd.) was used to evaluate each of the isolates for antibiotic resistance.

RESULTS

In bacterial profiling of LRTI among sanitary workers, both Gram positive and Gram negative bacteria were isolated and its morphology, motility and Gram nature indicated in Table 1. Table 2 shows that sugar fermenting capacity and biochemical characterization of isolated bacteria. The colonization and percentage of prevalent bacterial isolates on respective selective media was noted throughout the incubation period (Figure 1 and 2). This helps to identify the colonization nature of prevalent bacteria. Among the isolated bacteria, *S. pneumonia* (16%), Beta hemolytic streptococci (32%), *Staphylococcu aureus (S. aureus)* (25%), *Klebsiella* (50%), *P. aeruginosa* (65%) were observed predominantly.

The antibiotic sensitivity data of both Gram positive and Gram negative prevalent bacterial isolates represented in Table 3 and 4. This data suggests that prevalent microbes in LRT of sanitary workers are becoming sensitive to commonly used antibiotics, a great concern to medical fraternity. Though, out of five prevalent microbes, *P. aeruginosa* and *S. aureus* has shown remarkable resistance to the antibiotics (Table 3 and 4).

DISCUSSION

Our study is in good agreement with previous studies demonstrated that the infectious etiology of LRTI majorly associated with *P. aeruginosa, S. aureus, H. influenza* and *S. pneumonia* (Jacobs MR et al., 1994; Burman LA

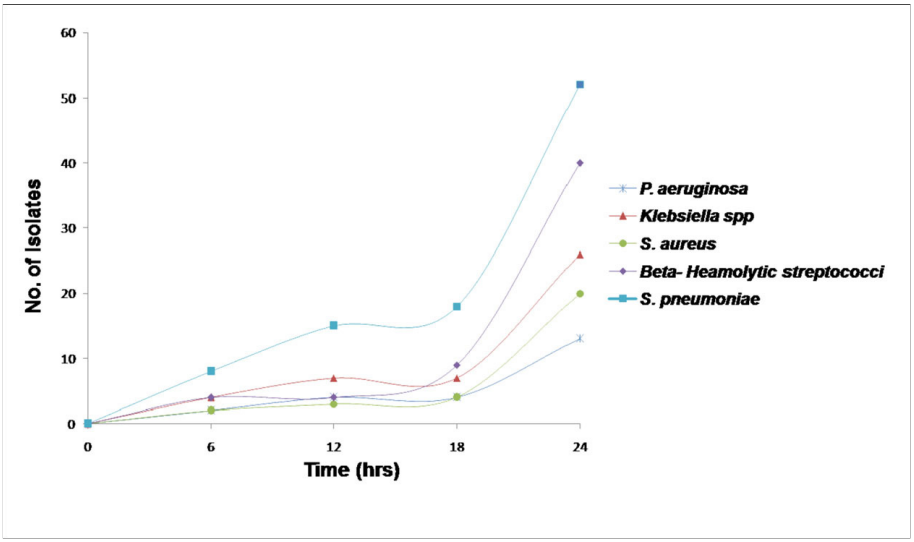

Figure 1. Colonization of prevalent bacterial isolates on respective selective media throughout the incubation period.

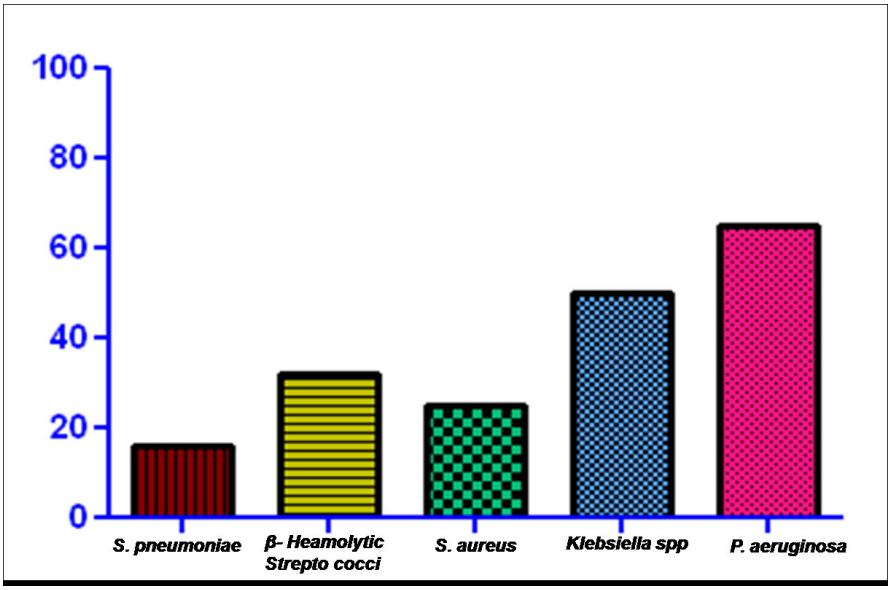

Figure 2. Percentage of prevalent bacterial isolates on respective selective media

Table 3. Prevalent Gram positive bacteria isolated from sanitary workers and their susceptibility to antibiotics

Prevalent Microbes	No. of Isolates (n=79)	Susceptibility to antibiotics (%)							
		A	C	E	P	T	CX	NA	CIP
S. pneumoniae	52	92.8	71.4	64.2	92.8	64.2	-	-	-
Beta hemolytic streptococci	40	43.7	81.2	81.2	87.5	42.7	-	-	-
S. aureus	20	28.5	85.7	57.1	14.2	42.8	-	-	-

Table 4. Prevalent Gram negative bacterial isolates from sanitary workers and their susceptibility to antibiotics

Prevalent Microorganisms	No. of Isolates (n=79)	Susceptibility to antibiotics (%)				
		A	T	G	CF	NX
Klebsiella spp	26	70	90	90	100	100
Pseudomonas spp	13	20	20	60	100	100

et al., 1991). Out of ten isolated bacteria, seven were catalase positive indicates that these microbes can able to survive in aerobic environment due to the defence against reactive oxygen species and oxidative stress (Table 2). Patients with CAP are having higher rates of *S. pneumonia*, ranging from 35% to 43% (Sethi S, 2000b). Even if, bacterial infection is the main cause of exacerbation in respiratory failure, the exacerbation does not entail bacterial infections inevitably (Friis L et al., 1999; Shakespeare A and Poole J, 1993).

Earlier, investigators reported that workers in organic dust environment with endotoxin exposure having increased airway problems (Rylander R and Jacobs RR, 1997; Thorn J and Rylander R, 1998). Further, an increased prevalence of COPD exacerbation and asthma has been reported among sewage workers (Jacobs MR et al., 1994).However, a definite causative link of bacterial colonization and exacerbation is unclear, therapy is in fact often empirical (Wubbel L et al., 1999; Gold HS and Moeelering, RC, 1996). We observed that bacterial isolates in LRT of sanitary workers were sensitive to commonly used antibiotics. Although *S. aureus* and *P. aeruginosa* has been shown significant resistant to the antibiotics, these data may be an imperative aid in deciding and formulating a correct antibiotic therapy. Pharmacodynamic studies imply that efficacy of antibiotics is suboptimal against the pathogens *invivo* (Anthonisen NR et al., 1987). Apparent clinical effects with persistence of pathogens may contribute to the development of resistance, relapse or repeat exacerbations.

CONCLUSION

Summarizing, sanitary workers are more frequently exposed and affected by diverse microbial population, particularly respiratory pathogens. The prevalence of *P. aeruginosa, S. aureus, S. pneumonia, Beta heamolytic Streptococci* and *Klebsiella* were found to be higher in the community of sanitary worker's lower respiratory tract. This type of infection induces impairment of local defence mechanism and facilitates microbial colonization in the respiratory airways. *S. aureus* and *P. aeruginosa* were shown remarkable resistance to the antibiotics which may help in finding new modalities of prevention and treatment for the infections among sanitary workers.

REFERENCES

Anthonisen NR, Manfreda J, Warren CP, Hershfield ES, Harding GK, Nelson NA (1987). Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. Ann. Int. Med. 106: 196-204.

Burman LA, Trollfors B, Andersson B, Henrichsen J, Juto P, Kallings I, Lagergard T, Mollby R, Norrby R (1991). Diagnosis of pneumonia by cultures, bacterial and viral antigen detection tests, and serology with special reference to antibodies against pneumococcal antigens. J. Infect. Dis. 163: 1087– 1093.

Craig WA (2001). Re-evaluating current antibiotic therapy. Respiratory Medicine. 95: S12–S19.

Ejlertsen T, Thisted E, Ebbesen Murphy TF, Sethi S (1992). Bacterial infection in chronic obstructive pulmonary disease. Am. Rev. Respir. Dis. 146: 1067–1083.

Friis L, Norbäck D, Edling C (1999). Self-reported asthma and respiratory symptoms in sewage workers. J. Occup. Health. 41: 87–90.

Gold HS, Moeelering, RC (1996). Antimicrobial drug resistance. N. Engl. J. Med. 335:1445-1453.

Jacobs MR, Bajaksouzian S, Zilles A, Lin G, Pankuch GA, Appelbaum PC (1994). Susceptibilities of *Streptococcus pneumonia* and *Haemophilus influenzae* to 10 oral antimicrobial agents based on pharmacodynamic parameters: 1997 U.S. surveillance study. Antimicrobial Agents and Chemotherapy. 43: 1901–1908.

Lusuardi M, Capelli A, Di Stefano A, Zaccaria S, Balbi B, Donner CF (2003). Lower respiratory tract infections in chronic obstructive pulmonary disease outpatients with tracheostomy and persistent colonization by P. aeruginosa. Respir. Med. 97: 1205-1210.

Melbostadt E, Eduard W, Skogstad A, Sandven P, Lassen J, Sostrand P, Heldal K (1994). Exposure to bacterial aerosols and work-related symptoms in sewage workers. Am. J. Ind. Med. 25: 59–63.

Rylander R, Jacobs RR (1997). Endotoxins in the environment: a criteria document. Int J. Occup. Environ. Health. 3: S1-48.

Schwartz DA, Thorne PS, Yagla SJ, Burmeister LF, Olenchock SA, Watt JL, Quinn TJ (1995). The role of endotoxin in grain dust induced lung disease. Am. J. Respir. Crit. Care Med. 152: 603-608.

Sethi S (2000b). Infectious exacerbations of chronic bronchitis: Diagnosis and management. J. Antimicrob. Chemotherapy. 43: 97–105.

Sethi S (2001a). Infectious etiology of acute exacerbations of chronic bronchitis. Chest. 117: 380S-385S.

Shakespeare A, Poole J (1993). Sewage workers and hepatitis A. Occup Health. 45: 364–366.

Thorn J, Beijer L, Rylander R. Work related symptoms among sewage workers: a nationwide survey in Sweden (2002). Occup. Environ. Med. 59: 562-566.

Thorn J, Rylander R (1998). Inflammatory response after inhalation of bacterial endotoxin assessed by the induced sputum technique. Thorax. 53: 1047-1052.

Wubbel L, Muniz L, Ahmed A, Trujillo M, Carubelli C, McCoig C, Leinonen M, McCracken GH Jr (1999). Etiology and treatment of community acquired pneumonia in ambulatory children. Pediatr. Infect. Dis. J. 18: 98-104.

Zuskin E, Mustafbegovic J, Schacter EN (1993). Respiratory function in sewage workers. Am. J. Ind. Med. 23: 751–761.

Zuskin E, Mustajbegovic J, Lukenda-Simovic D, Ivankovic D (1990). Respiratory symptoms and ventilatory capacity of sewage canal workers. Lijec Vjesn. 112: 353-357