ORIGINAL RESEARCH PAPER

Microbiology

MULTIPLE-DRUG RESISTANCE IN GRAM NEGATIVE BACTERIA ISOLATED FROM UPPER RESPIRATORY TRACT.

KEY WORDS: Multiple drug resistance, nosocomial infections

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ABSTRACT

There is a high prevalence of multiple drug-resistant (MDR) strains which has resulted due to the over use of antibiotics today. Antimicrobial resistance in nosocomial infections is increasing with both morbidity and mortality greater when drug resistant organisms cause the infection. Nosocomial infections are typically exogenous, the source being any part of the hospital ecosystem. Bacteria causing these infections are opportunistic and can cause disease in hospitalized patients whose immune mechanisms are impaired. Many antibiotics can no longer be used for the treatment of infections caused by such organisms. MDR infections are often significantly harder and more expensive to treat, hence represent a growing public health threat and challenge in medicine. Widespread antibiotic resistance and longer survival in the hospital environment has enhanced opportunities for transmission of such pathogens in patients.

Authorities have been strongly encouraging physicians to decrease the prescription of antibiotics to treat common upper respiratory tract infections. In addition, as viruses mainly cause these infections, antibacterial antibiotics do not significantly help in reducing the recovery time of the illness. Rather the overuse of antibiotics has proved to be a major factor in the emergence and dissemination of multi-drug resistant strains (Harbottle et al., 2006). The worldwide emergence of ß-lactamase producing Escherichia coli, Klebsiella pneumoniae, Haemophilus and many other strains have currently become a major therapeutic problem. They are resistant to the -lactam antibiotics namely penicillins, cephalosporins and some carbapenems. These strains are increasingly being isolated from hospital and community acquired infections (Khan 2004, Harbottle et al., 2006, Zsuzsanna et al., 2010).

As most of the URTIs are self-limiting, do not require the use of antibiotics. Many times, adequate amount of fluids, antipyretic drugs as symptomatic treatment for cough and cold can help to recover from these infections (Wong D et al., 2006). Literature shows that symptoms improve after treatment with -adrenergic agonists and anti- histamines. Warm saline gargles and steam inhalation are inexpensive and relatively safe measures that also provide temporary relief of throat symptoms. Nonsteroidal anti-inflammatory drugs prove to be useful for relieving fever, headache, and malaise (Wong D et al., 2006).

Commonly found Gram negative organisms associated with URT belong to the species of

1. Klebsiella species as a clinically significant opportunistic bacterial pathogen that can infect immune compromised individuals who are hospitalized. Klebsiella ozone, another member of this genus known to cause "ozena", a primary atrophic rhinitis (AR) which involves chronic inflammation of the nose. Klebsiella rhinoscleromatis another pathogen of the genera causes rhinoscleroma (RS), a chronic granulomatous infection, which predominantly affects the cavity of the nose (Janda and Abbott, 2006).

2. Pseudomonas aeruginosa rarely causes community acquired pneumonia. The result of its ability to produce different lactamase is the most frequent pathogen involved in acute exacerbation of chronic bronchitis.

3. Acinetobacter baumannii occasionally a normal habitant of the oropharynx is commonly isolated from hospitalized patients. It grows in moist environments like the respiratory tract instruments in hospitals. Acinetobacter infections are uncommon but, it usually involves organ systems that have a high fluid content (e.g., Respiratory tract, CSF, peritoneal fluid). This organism is seen in ventilator associated pneumonia in patients with respiratory-support equipment or fluids.

4. Fusobacterium necrophorum -Normal inhabitants of the oropharyngeal flora can cause peritonsillar abscess near the tonsils after streptococcal sore throat.

5. Helicobacter spp- It is suggested that H. pylori enters the nasopharyngeal cavity by gastro-oesophageal reflux and colonize in dental plaques, adenoid tissues and tonsils. From these localizations, the bacteria ascend to the middle ear and to the para nasal sinuses directly or by the reflux again and may trigger some diseases, including otitis, sinusitis, pharyngitis, laryngitis and glotitis. (Kurtaran et al., 2008).

The worldwide emergence of ß-lactamase producing Escherichia coli, Klebsiella pneumoniae, Haemophilus and many other strains have currently become a major therapeutic problem. They are resistant to the -lactam antibiotics namely penicillins, cephalosporins and some carbapenems. These strains are increasingly being isolated from hospital and community acquired infections (Khan 2004, Harbottle et al., 2006, Zsuzsanna et al., 2010). Rather the overuse of antibiotics has proved to be a major factor in the emergence and dissemination of multi-drug resistant strains (Harbottle et al., 2006).

The spontaneous mutation frequency of antibiotic resistance is a rare event, in the order of about of about 10-8- 10-9. Nevertheless, high growth rate of bacteria results in considerable resistance developed in a population. In vertical transfer, there is a direct transfer of resistance genes to all the bacterial progeny during DNA replication. The genetic determinants encoding antimicrobial resistance can be located on the bacterial chromosome or on plasmids, which may replicate independently of the chromosome.

Lateral or horizontal gene transfer (HGT), the resistance-plasmid is transferred between individual bacteria of the same species or even between different species, which spread resistance within a population. This acquisition of the genetic determinants takes place by the process of conjugation, transduction and transformation (Tenover FC. 2006; Jayaraman R 2009).

MDR or Multiple drug resistance is a condition enabling the microorganism to resist the inhibition by antimicrobials of a wide variety of structure and function targeted at eradicating the organism. Though it is a common term associated with Mycobacterium infections, it generally refers to those organisms that show resistance to more than one family of antibiotic. These bacteria show to accumulate multiple genes, each coding for resistance to a single drug, within a single cell. This accumulation occurs typically on resistance (R) plasmids. There is an increased expression of genes that code for multidrug efflux pumps, extruding a wide range of drugs.

Such strains also show a decrease in outer membrane permeability through mutations in Porin genes (Tenover FC.2006;Nikaido H.2009).

METHODOLOGY

1.300 samples were collected include throat swabs, nasal and para-nasal swabs, Ear exudates Tonsillectomy/tonsil biopsies, Oral swabs, Sinus drains

Inclusion Criteria: Patients referred from the outpatient department, wards, Intensive care units and operating theatres, clinically suspected to have an upper respiratory infection and showing suspicion of infective etiology. All age groups and both the sexes were considered.

Exclusion Criteria: Patients with symptoms of lower respiratory infections. Sputum samples, tracheal aspirates, bronchial biopsies were excluded as they represent lower respiratory infections. Ear exudates of patients showing symptoms of otitis externeae were eliminated

2.The analytical profile index or API system was used for quick identification of clinically relevant isolates after a prior confirmation.

3. Disc diffusion test a qualitative test method documented by The National Committee for Clinical Laboratory was used to test the antibiotic sensitivity (NCCLS). Antibiotic rings for Gram Negative- Dodeca Enterobacteriaceae-1 DE053- ring, Hi Media were used

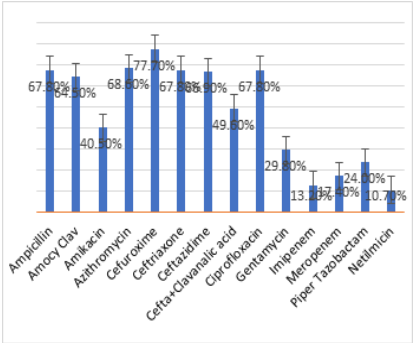
3.DNA was isolated from the Broth culture of the significant isolates. Electrophoresis in a 1% Agarose gel and visualized under UV. The 16S r DNA gene was PCR amplified with 16S forward and reverses primers. Amplicon was electrophoresed in a 1% Agarose gel and visualized under UV. Con-centration of the amplicon was checked in a Nanodrop ND 2000.

RESULTS

Table 1: Distribution of pathogenic species

Culture	No.	Percentage
Klebsiella spp	64	34.4
Pseudomonas spp	41	22.04
Acinetobacter spp	13	6.98
E. coli	2	1.07
Proteus spp	1	0.05
Streptococcus pyogenes	30	16.12
Candida albicans	17	9.13
Staphylococcus aureus	12	6.45
Micrococcus spp	5	2.68
Corynebacterium diphtheriae	1	0.05
Total number of Isolates: n = 186		

Graph 1: Antibiotic sensitivity of Gram-negative isolates



Gram negative isolates, which showed resistance towards multiple antibiotics were further, identified using 16 s RNA sequencing. As these isolates were collected from patients in ICU, they represent hospital acquired infections. Their sensitivity to herbal extracts can be exploited for the effective control of such infections. Sequencing of DNA of the isolates was done in SciGenom Labs, Cochin SciGenome lab Cohin on ABi 3730Xl machine using the Sanger sequencing methodology

Table 2: 16 s RNA sequencing results

Isolate No	Sequence Used	Description	Accession Number
R016	R016a_27F_7864-7_5160, Raw Sequence (1228 bp)	Pseudomonas aeruginosa PAO1, complete genome	AE004091.2
R062	R62ps_16SF_6527-3_P1, Raw Sequence (877 bp)	Pseudomonas aeruginosa strain SK9 16S ribosomal RNA gene, partial sequence	KC790300.1
R265	R265kp_16SF_6527-1_P1, Raw Sequence (799 bp)	Acinetobacter baumannii strain OVC5 16S ribosomal RNA gene, partial sequence	JQ660721.1
R307	R307kp_16sF_6527_P1. Raw sequence	Klebsiella Sp. ANctcri2 16S ribosomal RNA gene, partial sequence	HQ286642.1
R562	R562kp_16SR_6527-2_P1, Raw Sequence (1209 bp)	Acinetobacter baumannii Naval-18 clone 1061064264440 16S ribosomal RNA gene, partial sequence	JN668115.1
R608	R608ps_16sR_6527-10_P1, Raw Sequence (522 bp)	Pseudomonas aeruginosa strain F23 16S ribosomal RNA gene, partial sequence	JQ579643.1
R610	R610a_27F_7864-7_5160, Raw Sequence (1228 bp)	Pseudomonas aeruginosa strain ALK320 16S ribosomal RNA gene, partial sequence	KC456535.1
R 635	R635.a_27F_7864-1_5160, Raw Sequence (1023 bp)	Stenotrophomonas maltophilia strain S-3 16S ribosomal RNA gene, partial sequence	JX868559.1
R703	R703kp_16SF_6527-7_P1, Raw Sequence (879 bp)	Klebsiella Sp. ii_2_chl_3 16S ribosomal RNA gene, partial sequence	JQ838154.1

DISCUSSION

The 16s r-RNA sequences of nine of the MDR isolates were analyzed using Bioinformatics tools like BLAST and Clustal omega, for their phylogenetic analysis for molecular confirmation and strain level identification. The 16 s rRNA studies confirmed the following isolates from clinical samples. These isolates isolated from patients in the intensive care units. They require special attention in terms of hospital care and hygiene to control Community acquired and nosocomial infections, which pose a threat to treatment in hospitals. Molecular identification in diagnostic microbiology is a vital need for the MDR strains which pose a threat in chemotherapy as potential pathogens need to be

identified and confirmed at molecular level for targeted treatment.

1. Stenotrophomonas (Pseudomonas) maltophilia was identified from an intensive care unit patient, an uncommon respiratory pathogen in humans. S maltophilia is noted to be naturally resistant to many broad-spectrum antibiotics (including all carbapenems) due to the production of two inducible chromosomal metallo- -lactamases. This makes treatment of infected patients very difficult. Waters et.al (2007) reported S. maltophilia to be ubiquitously found in the environment and impossible to eradicate, which makes prevention also extremely difficult. The increase in incidence of nosocomial and community-acquired S. maltophilia infections are of particular concern. It needs to be monitored especially in immune compromised individuals.
2. Pseudomonas aeruginosa was confirmed in of the clinical isolates.

i) Pseudomonas aeruginosa PAK strain reported by Vasseur et al., (2005), known to cause chronic bacterial infections in their scientific studies. They are associated with the bio film, lifestyle of the bacteria, wherein the micro colonies are embedded in an extracellular matrix. These strains possess the PEL genes, which encode proteins with similarity to components involved in polysaccharide diagnosis. These are involved in the production of a glucose-rich matrix material during the formation of a thick pellicle and resistant bio films that make them resist most of the antibiotic.

ii) Pseudomonas aeruginosa F3 strain on research by Raorane et al., (2012) was found to be degraded by Phthalic acid (a precursor used in the manufacture of plastic is the most frequent colonizer of medical devices, community-acquired pneumonias, as well as ventilator-associated pneumonias in immune compromised individuals. Clove was highly effective in restricting the growth of this strain

iii) Pseudomonas aeruginosa PAO1 is a common, ubiquitous metabolically versatile strain and opportunistic pathogen. A major factor in its prominence as a pathogen is its intrinsic resistance to antibiotics and disinfectant. It is proposed that size and complexity of the P. aeruginosa genome reflects as an evolutionary adaptation. This permits it to thrive in diverse environments and resist the effects of a variety of antimicrobial substances.

3. Acinetobacter baumannii noted opportunistic pathogen in people with compromised immune systems is increasingly important as a hospital derived infection. A. baumannii is part of the ACBcomplex (A. baumannii, A. calcoaceticus, Acinetobacter genomic species 13TU). Members of the ACB complex are difficult to speciate (to determine the specific species). AbaR-type resistance islands are typical of MDR was identified as one of the isolates in the present survey. Seputiene et al., (2012) have shown the presence of resistant genes provide resistance to antibiotics such as Aminoglycosides, Aminocyclitols, Tetracycline, and Chloramphenicol which are commonly used to treat respiratory infections.
4. Klebsiella pneumoniae is a pathogen in a significant proportion of hospital-acquired urinary tract infections, pneumonia and septicemias. Hospital outbreaks of multidrug-resistant Klebsiella spp., especially those in intensive care wards, are often caused extended-spectrum-beta-lactamase (ESBL) producers. The incidence of ESBL-producing strains among clinical Klebsiella isolates has been steadily increasing over the past years. These strains have the ability to utilize Chloramphenicol as the sole source of carbon and energy and thereby resist the action of most of the antibiotics. Result on bioactivity of Clove to restrict these strains was consistent with the result is previously cited by (Saeed et al., 2008; Jennifer et al. 2009; Ayoola et al., 2008).

CONCLUSION

The present study provides evidence of viral etiology in 53.8% of upper respiratory infections. This can be taken into consideration to decide the line of treatment of upper respiratory infections. It has proved that antibiotic-resistant strains of pathogenic bacteria are increasingly prevalent in hospital community with maximum drug resistance shown by Klebsiella spp and Pseudomonas spp.

Historically, most antibiotics are developed from a small set of molecular scaffolds whose functional lifetime have been extended by generations of synthetic tailoring. Development of resistance in 31.18% of ESBL to cephalosporin and 15.59% multidrug resistant isolates suggests that the discovery of new scaffolds should be a priority.

The current findings add substantially to our understanding that new antibiotics are needed to combat these bacterial pathogens. Progress in developing them has been slow, as a dramatic reduction in antibiotic research by pharmaceutical companies is seen due to the high cost of drug research.

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