Prevalence of Antibiotic Resistance in Gram Positive Bacteria Related to Upper Respiratory Infections

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Abstract

The introduction and increasing use of antibiotics for antibacterial therapy has initiated a rapid development and expansion of antibiotic resistance in microorganisms, particularly in human pathogens. Additionally, a shift to an increase in the number and severity of Gram-positive infections has been observed the last decades. Common to these pathogens is their tendency to accumulate multiple resistances under antibiotic pressure and selection. *Staphylococcus* spp. and *Streptococcus* spp. are the most common Gram-positive pathogens associated with upper respiratory tract infections. *Methicillin-resistant Staphylococcus aureus* (MRSA), that have acquired multi-resistance to all classes of antibiotics, have become a serious nosocomial problem. Problems with multi resistance expand to penicillin-resistant *Streptococcus pneumoniae* that is partially or totally resistant to multiple antibiotics, and to Vancomycin-resistant *Enterococcus* spp. The rapid development of resistance is due to mutational events, gene transfer and acquisition of resistance determinants, allowing strains to survive antibiotic treatment.

KEYWORDS: Respiratory infections, Antibiotic resistance, Nosocomial infections.

INTRODUCTION

Clinical symptoms of upper respiratory infections are highly variable and cannot be used to identify the etiologic agent or agents. Although the majority of respiratory infections is caused by a virus (69%), antibiotics are widely prescribed to treat symptoms.

Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases (*Jain 2001).* Antibiotics are of little or no benefit for viral infections, contribute to the emergence and spread of resistant bacteria and higher costs for health care. Overuse of antibiotics is also the major factor in the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms **(***Michael 2007*, *Harbottle 2006).* The worldwide emergence of *Streptococci, Escherichia coli, Klebsiella pneumoniae, Haemophilus* and many others ß-lactamase producers have become a major therapeutic problem. These strains are widely distributed in hospitals and are increasingly being isolated from community acquired infections. Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Enterococci* (VRE) are of particular concern in respiratory infections. *Streptococcus*, *Haemophilus, Moraxella,* and *Staphylococcus* species are usually found as normal inhabitants of the upper respiratory tract, which occasionally turn into pathogens causing infectious diseases. Other bacteria, such as *Pseudomonas*

spp, Klebsiella spp and Acinetobacter are also recently found to be associated with upper respiratory infections as secondary invasion by these bacterial pathogens is very common *(Zsuzsanna Schelz 2010).* The incidence of nosocomial *Candidemia* is also on the rise in the last decade.

Health authorities have been strongly encouraging physicians to decrease the prescribing of antibiotics to treat common upper respiratory tract infections because an antibiotic usage does not significantly reduce recovery time for these viral illnesses **(***Rice, 2006).* Rather antibiotic resistance is further aggravated by the horizontal spread of resistance genes between these bacterial species. The increasing rate of antibiotic resistance of bacteria urges new attempts to overcome the problem. Therefore antimicrobial agents with new mechanisms or resistance modifiers of synthetic and natural origin serve an alternative way of antimicrobial chemotherapy.

Role of Gram positive bacteria in respiratory infections

Bisno (2001) has shown 15% of acute pharyngitis cases are most commonly caused by *Streptococcus pyogenes* (GAS) resulting in tonsillitis commonly referred to as "*Strep throat"*. The cell surface of *Streptococcus pyogenes* accounts for the determinants of virulence, especially those concerned with the colonization and evasion of phagocytosis and the host immune responses. Asymptomatic nasopharyngeal carriage of *Streptococcus pneumoniae* or *Pneumococci* is widely prevalent among young children and is an important factor in the development and transmission of pneumococcal disease. In reports by *Fedson (1994)* nearly 54% of children were found carrying *Pneumococci* in their naso-pharynx by the time they are a year-old. Nasopharyngeal carriage may occur in 60% of healthy preschool children and 30% of older children and in adults. *Streptococcus* spp. can evade the host defence in normal and impaired hosts and spread to the upper or lower respiratory tract. These results in infections such as acute otitis media (AOM), sinusitis and pneumonia, or it may invade the bloodstream, causing invasive diseases.

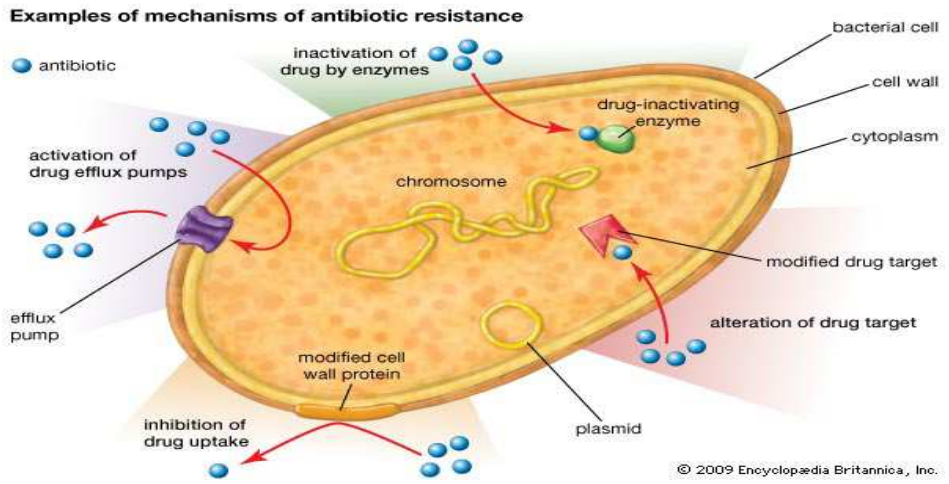
Staphylococcus aureus are frequently found in the human respiratory tract and on the skin, in the anterior nares of the nasal passages. It has been reported as a successful pathogen by *Greenberg (2004)* due to a combination of nasal carriage and bacterial immune-evasive strategies. *Whitby (2010)* has shown MSSA (Methicillin-sensitive *Staphylococcus aureus*) is more commonly isolated than MRSA from sinus cultures. As a relatively high (25-30%) percentage of adults who have MSSA colonized in the nasal passage, only about 2% of healthy people carry MRSA in the nose without any symptoms. The majority *Staphylococcus aureus* cultures isolated were mixed with other respiratory pathogens, principally *Haemophillus influenzae.* Case studies have revealed *Staphylococcus aureus* strains colonize up to 35% of young children and are associated with a wide range of diseases including soft tissue infections, sepsis, and pneumonia. Increases in the incidence of disease caused by community-acquired methicillin-resistant *Staphylococcus aureus* make the treatment of these infections difficult **(***Crum 2006).*

Common mechanism of Antibiotic resistance

The mechanisms of antibiotic resistance in bacteria can be broadly listed as:

i) Inhibition of uptake of the antibiotic ii) Efflux pumps are high-affinity reverse transport systems located in the membrane that transport the antibiotic out of the cell.

This is the mechanism of resistance to tetracycline. iii) A specific enzyme modifies/inactivates the antibiotic in a way that it loses its activity. In the case of streptomycin, the antibiotic is chemically modified so that it will no longer bind to the ribosome to block protein synthesis. iv) An enzyme is produced that degrades the antibiotic, thereby inactivating it. For example, the penicillinases are a group of beta-lactamase enzymes that cleave the beta lactam ring of the penicillin molecule.



Source: https://www.britannica.com-multiple mechanisms by which bacteria can develop resistance

METHODOLOGY

The present study was conducted in the Department of Microbiology, T. N. Medical College during January–June 2013.Samples from patients of all age groups, patients who were clinically suspected to have a respiratory infection and showing suspicion of infective etiology was considered. A brief, relevant history was noted to ensure that they were not administered with antibiotics in the past 48 hrs. Samples accepted were throat swabs, nasal and para-nasal swabs, bronchial and tonsil biopsies, ear exudates, oral swabs and sinus drains. Exclusion criteria were healthy patients, contaminants and ear exudates of patients showing symptoms of oitits externeae were not considered.

Microscopy: All samples were stained by the Grams staining method to determine the presence of oro-pharyngeal contamination (indicated by squamous epithelial cells) and as well as to identify the most likely pathogens (Indicated by the predominant organisms associated with WBCs).

Culture processing: All samples were sub cultured on Sterile Nutrient agar, Blood agar, Mac-Conkeys agar; Quality assurance passed media where each batch was tested with ATCC strains was used. Culture plates were incubated at 37^{o}C for 24 - 48 hours, after which the results were recorded. The analytical profile index or API system was used for quick identification of clinically relevant isolates after a prior confirmation.

Antibiotic sensitivity testing: Disc diffusion test a qualitative test method documented by The National Committee for Clinical Laboratory was used to test the antibiotic sensitivity (NCCLS). This was carried out using the Kirby Bauer's

technique. Antibiotic discs from Hi -media laboratories of mentioned antibiotics and known concentrations were used along with testing of quality control strains of *E. coli* ATCC 25922 and Pseudomonas aeruginosa ATCC 27853. Dodeca-G-III Ring Containing antibiotic concentration of Penicillin G (P) 10 Unit, Ampicillin (AMP) 10 µg, Oxacillin (OX) 1µg, Erythromycin (E) 15µg, Co-Trimoxazole (COT) 25µg, Vancomycin (VA) 30µg, Cefotaxime (CTX) 30µg was used.

Confirmation of MRSA: Oxacillin screen agar test: A saline suspension of the isolate (turbidity identical to0. 05 McFarland tube) was spotted on MHA Plate containing 6mcg/ml of Oxacillin and 4% NaCl. Any visible growth on incubation at37^{o}Cfor 24-48 hours was indicative of resistance. *S. aureus* ATCC 25923 (mec-A negative) and ATCC 43300 (mec-A positive) were used as controls for all the tests (*National Committee for Clinical Laboratory Standards).*

RESULTS

This Cross-sectional observational study was conducted to determine the clinical profiles of drug-resistant isolates prevalent in respiratory infections. Results with the help of all the information were recorded. Wherever results were recorded in triplicate an average was considered for final calculation.

The observations and results were subjected to Analysis of variance (ANOVA), so as to study the prevalence of multi drug resistance to various antibiotic families and development of resistance patterns and incidences of Hospital acquired infections in hospital, wards. SPSS Version 17 was used for most of the analysis.

The qualitative data were represented in the form of frequency and percentage, whereas quantitative data were represented using mean ± S.D. The results were graphically represented where deemed necessary. Distribution of 186 clinical isolates is shown in Table No: 1.

Table No.-1: Distribution of clinical isolates.

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
Coryn. diphtheriae	1	0.05
Total number of Isolates: n = 186		

Graph No.-1: Distribution of clinical isolates

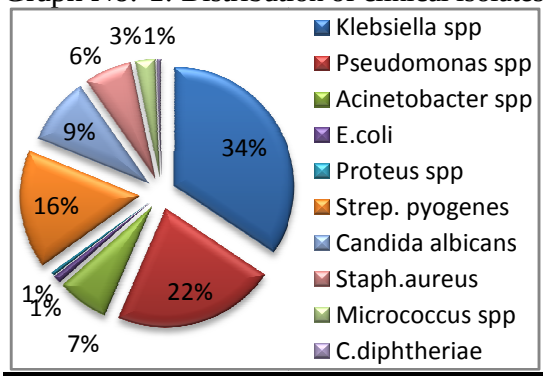


Table No: 2: Distribution of Gram positive isolates collected

LOCATION	OPD	WARDS	ICU	MICU	OTHERS
Streptococcus pyogenes (*n*=30)	04	21	03	--	02
Staphylococcus aureus (*n*=12)	05	06	--	01	--
Micrococcus spp. (*n*=5)	02	02	--	--	01

On studying the resistance pattern of the Gram positive cocci (Table No-3; Graph No-2) maximum resistance was seen towards Ampicillin, and the most effective antibiotic was Vancomycin (14.9%). Detailed distribution and antibiotic sensitivity profile of the three Gram positive cocci is shown in Table No-4 and Table No-5.

Table No.3: Resistance seen in Gram positive isolates

Antibiotic	% of Resistant Isolates
Penicillin (P)	36.2%
Ampicillin (AMP)	59.6%
Cefotoxime (CTX)	44.7%
Erythromycin (E)	44.7%
Vancomycin (VA)	14.9%
Oxacillin (OX)	34.0%
Co-trimaxole (COT)	14.9%

Graph No.: 2: Percentage of Resistance to Antibiotics

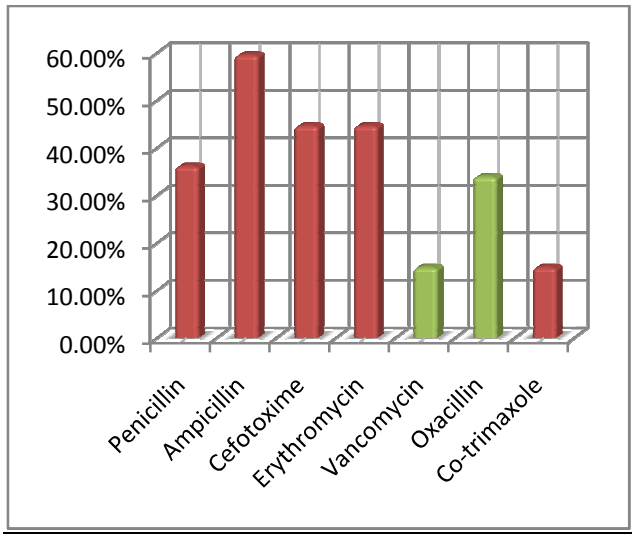


Table No.:4: Bacterial species wise resistance towards antibiotics

ISOLATED SPECIES		Sensitivity of isolates towards antibiotics						
		P	AMP	CTX	E	VA	OX	COT
Streptococcus pyogenes n=30	R	16	04	04	10	02	04	02
	I	10	03	06	--	--	01	02
	S	09	21	20	02	22	25	26
Staphylococcus aureus n=12	R	05	02	06	06	04	07	02
	I	03	03	01	--	--	--	--
	S	04	07	04	06	08	05	10
Micrococcus spp n=5	R	--	--	--	--	--	--	--
	I	--	01	--	01	--	--	--
	S	05	04	05	04	05	05	05

Table No 5: Distribution of *Staphylococcus* isolates

No. Of Strains Isolated	CONS	MRSA/ ORSA	VRSA	OTHERS
n=12	5	7	3	14

KEY: CONS: Coagulase negative Staphylococcus; MRSA Methicillin resistant Staphylococcus aureus; VRSA –Vancomycin resistant Staphylococcus aureus

Staphylococcus strains were subjected to Methicillin resistance tests and results recorded (Table No -5), seven were identified as MRSA as they showed resistance to

methicillin and other drugs in the same class, including Penicillin, Amoxicillin, and Oxacillin. It also showed resistance to cephalosporin. MRSA can be termed as a "superbug", as it is a strain of bacteria that has become resistant to the antibiotics usually used to treat it. The occurrence of these strains was seen from hospital wards in patients showing respiratory infections indicate its presence in hospitals. In such settings; MRSA is referred to as health care-associated MRSA (HA-MRSA). Methicillin-resistant *Staphylococcus areas* have also been noted to be the causative pathogen in 15-27% of ventilator-associated pneumonia and healthcare-associated pneumonia (*Chang 2011).*

MRSA is usually found in community associated because of sharing contaminated items, having active skin diseases or injuries, poor hygiene, and living in crowded settings. The transmission of MRSA is largely from people with active MRSA skin infections and is spread by direct physical contact and not through the air. Spread can also occur through indirect contact by touching objects (such as towels, sheets, wound dressings, clothes, workout areas, sports equipment) contaminated by the infected skin of a person with MRSA in hospital wards. Three strains of *Staphylococcus* have been identified that are resistant to antibiotic Vancomycin which is normally effective in treating Staphylococcal infections. These strains are referred to as Vancomycin-resistant *Staphylococcus aureus* (VRSA).

DISCUSSION

The occurrence of 58.3% of *Staphylococcus aureus* isolates exhibited resistance to methicillin (MRSA) draws attention. These are strains of *Staphylococcus aureus* that has developed, through the process of natural selection, resistance to β -lactam antibiotics, which include penicillins (methicillin, dicloxacillin, oxacillin, etc.) and the cephalosporin. As the samples were collected from hospital wards, outbreaks of hospital-acquired MRSA (HA-MRSA) are typically the result of the clonal spread of MRSA being transferred from patient to patient, frequently using health care personnel as intermediaries. Resistance to β-lactam antibiotic drugs in these strains are mediated by alterations in PBPs (Penicillin binding proteins).

Vancomycin is the next standard treatment for serious MRSA infections, but a few (33.3%) cases of Vancomycin-resistant *Staphylococcus aureus* (VRSA) have recently emerged **(***Appelbaum2007).* In the present study the occurrence of 14.9% of Vancomycin resistance amongst all of Gram positive the clinical isolates along with three VRSA draws serious attention. Vancomycin remains first-line antimicrobial therapy for serious infections caused by MRSA and is available in multiple generic formulations; Vancomycin is reasonably well tolerated, associated with a low incidence of adverse effects, and relatively inexpensive. However, despite being the criterion standard therapy, the susceptibility of MRSA to this antibiotic may be decreasing, and reports of clinical failure are increasing.

Emergence of resistance to β-lactam antibiotics in clinical isolates of *Streptococcus* was also observed. This is a result of increasing frequency of decreased affinity towards the antibiotic in this species. Résistance to Erythromycin in 33% of *Streptococcal* species could be explained as Macrolide resistance as a result of modification of the target site by plasmid coded proteins. Penicillin is the drug of choice for *Streptococcal* pharyngitis. Allergic reactions to Penicillin has resulted the use of macrolide antibiotics like Erythromycin and Azithromycin. Over consumption of these antibiotics has resulted in the development of this resistance. Though resistance to Vancomycin was seen in 6% of *Streptococcal* species, Vancomycin (glycopeptide antibiotic) is the next drug of choice in such cases. *New options for*

treatment of resistant infection: Ceftaroline, Linezolid, Telavancin.

REFRENCES:

Ana Maria Rivera, MD and Helen W. Boucher, (2011). Current concepts in antimicrobial therapy against select Gram-positive organisms: Methicillin-Resistant *Staphylococcus aureus*, Penicillin-Resistant *Pneumococci*, and Vancomycin-Resistant *Enterococci.* Mayo Clin Proceedings held in December 2011; 86 (12): 1230–1243.

Appelbaum PC (2007). Reduced glycopeptide susceptibility in methicillin-resistant *Staphylococcus aureus* (MRSA). Int. J. Antimicrob. Agents 30 (5): 398–408

Bisno AL (2001). *Acute pharyngitis*. N. Engl. J. Med; 344: pp205.

Chan JD, Pham TN, Wong J. (2011). Clinical outcomes of linezolid vs vancomycin in methicillin-resistant *Staphylococcus aureus* ventilator-associated pneumonia: A retrospective analysis. Journal of Intensive Care Med.26pp:385-391.

Crum NF, Lee RU, Thornton SA, Stine OC, Wallace MR, Barrozo C. (2006). Fifteen-year study of the changing epidemiology of methicillin-resistant Staphylococcus aureus. Am J Med.;119 (11): pp943-51.

Fedson DS, Musher DM. (1994). *Pneumococcal* vaccine. Prevention pneumococcal Disease: Recommendations of the advisory committee on Immunization practices (ACIP). J. Inf. Dis., 170: pp 368–376.

Greenberg, D. (2004). Relative importance of nasopharyngeal versus oropharyngeal sampling for isolation of *Streptococcus pneumoniae* and *Haemophilus influenzae* from healthy and sick individuals varies with age. J. Clin. Microb. 42: pp 4604–4609.

Harbottle H., Thakur, S., Zhao, S., White, D.G. (2006). Genetics of Antimicrobial Resistance, Anim. Biotechnol- *17*: pp111-124.

Jain N, Lodha R, Kabra SK. (2001). Upper respiratory tract infections**.** Indian J. Paediatrics. 68 (12): pp1135-8.

Michael J. Lodes, Dominic Suciu, Jodi L. Wilmoth (2007). Identification of Upper Respiratory Tract Pathogens Using Electrochemical Detection on an Oligonucleotide Microarray. PLoS ONE 2 (9): e924.

National Committee for Clinical Laboratory Standards -Performance standards for antimicrobial disk susceptibility tests. 8th Ed.

Rice L.B (2006). *Antimicrobial* resistance in Gram-positive bacteria. Am J MED.; 119 (6 Suppl 1): 62-70.

Whitby C, Kaplan S, Mason E (2010). *Staphylococcus aureus* - Sinus Infections in Children. Int J of Ped Otorh: pp 118-121.

Zsuzsanna Schelz, Judit Hohmann, and Joseph Molnar (2010). Recent advances in research on antimicrobial effects of essential oils and plant derived compounds on bacteria. Ethnomedicine: A Source of Complementary Therapeutics, 179-201.