exhibited a potent post-antibiotic effect when compared to Vancomycin. In order to elucidate their mechanism of action, resistant mutants with a MIC of 64 mg/L were generated (frequency  $\sim\!10^{-7})$  and are being characterized at the molecular level to decipher mechanism of action of these compounds.

**Conclusion**: A series of 2-Aryl indole-based 2,3-epoxy-1,4-naphthoquinones have been synthesized with potent anti-MRSA activity. These compounds potentially deplete thiols, thus enhancing ROS in bacteria, which might help in overcoming drug resistance.

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## Susceptibility pattern of healthcare-associated methicillin resistant staphylococcus aureus to Vancomycin and Daptomycin



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**Background:** Healthcare-associated methicillin resistant *Staphylococcus aureus* (HA-MRSA) is a major pathogen. Vancomycin is used in the treatment of serious infection caused by HA-MRSA. However, the emergence of Vancomycin intermediate *S.aureus* (VISA) and vancomycin resistant *S.aureus* (VRSA) is a matter of concern. The present study was conducted to determine minimum inhibitory concentration (MIC) of vancomycin and daptomycin among HA-MRSA in our healthcare settings.

**Methods & Materials**: A total of 110 HA-MRSA isolates (as defined by Centres for Disease Control and Prevention criteria) were collected over a period of seventeen months. Vancomycin MIC was determined by agar dilution method according to CLSI guidelines. Daptomycin MIC was determined by E-test (BioMerieux, France).

**Results**: Out of the total HA-MRSA isolated, 53.6% (59/110) had vancomycin MIC of  $2\mu g/ml$ . Intermediate resistance to vancomycin was detected in 03.6% (04/110) of the HA-MRSA and these isolates had a vancomycin MIC of  $4\mu g/ml$ . All HA-MRSA isolated were sensitive to daptomycin.

**Conclusion**: Occurrence of VISA among the HA-MRSA is a matter of concern. As these strains do not respond to vancomycin treatment hence their detection is crucial. VISA cannot be detected by routine disk diffusion method. Determination of MIC of vancomycin is necessary for the detection of VISA. Daptomycin can be effectively used in the treatment of infections caused by VISA isolates in our healthcare settings as resistance to this antibiotic has not yet been observed.

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Prevalence and antibiotic sensitivity of Staphylococcus aureus and Pseudomanas aeruginosa in middle ear fluids of chronic suppurative otitis media and chronic rhinosinusitis patients undergoing ear surgery



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**Background:** Chronic Suppurative Otitis Media (CSOM) and Chronic Rhinosinusitis (CRS) are strongly associated common diseases with a significant impact on people's quality of life worldwide. Emergence of antimicrobial resistance of the causative microbes poses problem in management of the disease. The present study aimed to find the microbial prevalence and compare the antibiotic sensitivity in middle ear fluid isolates of CSOM and CRS patients with CSOM in South Indian population.

**Methods & Materials**: 86 subjects with CSOM and 68 patients with CRS undergoing ear surgery at a MAA ENT Hospitals, Hyderabad, South India between 2009 and 2015 were included in the study. The middle ear aspirates were collected aseptically, cultured by conventional methods and tested for antibiotic sensitivity using Kirby Bauer disc diffusion method. Chi-square analysis was performed to test the difference between the two groups.

**Results**: The present study included 99 males and 57 females with mean age of  $34.06 \pm 19.215$  yrs. Significant difference in prevalence of bacterial isolates with respect to sex and age was seen between the two groups (table 1). The most frequent microbial isolates in CSOM subjects was Pseudomonas aeruginosa (24%) followed by Staphylococcus aureus (19%) whereas in CRS with CSOM subjects, Staphylococcus aureus was 45% and Pseudomonas aeruginosa was 20%. Antibiotic susceptibility of staphylococcus aureus was high to cefotaxime, amikacin and gentamicin in both the groups. Antibiotic resistance of staphylococcus to ciproflaxacin is 78.8% and vancomycin is 55% in CSOM subjects . High rate of antibiotic sensitivity of Pseudomonas aeroginosa was observed for imipenem, piperacillin tazobactam, cefotaxime and amikacin in both the subgorups. Antibiotic resistance of Pseudomonas aeroginosa to ciproflaxacin is 55%, Gentamicin 47.2% and Cefepime 50% in CSOM subjects. Antibiotic resistance of Pseudomonas aeroginosa was not seen in CRS with CSOM subjects (Table 2).

Sex	CSOM (n)		CRS with CSOM		
	n	(n%)	n	(n%)	p value
Sex	47	64.6	37	54.4	
Male	21	24.4	26	38.24	
Female	26	30.2	11	16.18	0.016
Age					
0-10	4	4.7	12	17.65	0.01
10-20	23	26.7	9	13.24	
20-30	13	15.1	6	8.82	
30-40	12	14.0	11	16.18	
40-50	12	14.0	10	14.71	
50-60	5	5.8	5	7.35	



**Conclusion**: The antibiotic sensitivity of the common microbes differed significantly between CSOM and CRS with CSOM subjects in South Indian Population. The present study warrants the need for evaluation of antimicrobial susceptibility profile of the causative microbial pathogens before administration of antibiotics to treat CRS with CSOM in particular.

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## The emergence of cotrimoxazole and quinolone resistance in Shigella sonnei



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**Background**: The emergence of cotrimoxazole resistance has been a dominant and consistent character in our isolates of Shigella sonnei. To study the behaviour of these emerging strains and characterise mechanisms of resistance to cotrimoxazole & ciprofloxacin the following study was performed.

Methods & Materials: Isolates of Shigella sonnei confirmed by standard methods from 2012 to 2015 were subjected to antimicrobial susceptibility testing using the Kirby Bauer method as per Clinical laboratory standards institute and PCR for the detection of virulence genes. The degree of relatedness between the isolates was assessed by ERIC PCR followed by gel image analysis. Dendrogram was generated using Pyelph. PCR was carried out to determine the mechanisms of resistance to cotrimoxazole and ciprofloxacin.

Results: Of 34 Shigella sonnei isolates, cotrimoxazole resistance was common (94.1%) followed by ciprofloxacin (47%). Majority carried the ipaH gene (97%) followed by ial (17.6%), sen (11.7%), set 1 & set2 (5.8%). No stx element was found. ERIC-PCR analysis of the isolates resulted in four major ERIC groups labelled Eric group I,II,III and IV. Type III was the dominant (44.1%) type. Majority harboured dhfr1 (94.1%), sul2 (85.2%) followed by sul3 (55.8%), sul1 (11.7%). Two isolates that were resistant to cotrimoxazole were negative for the sul genes but harboured the dhfr1 gene. All the phenotypically ciprofloxacin resistant isolates (47%) were positive for presence of gyr A,gyr B, parC and parE. Also, qnrB was the most prevalent PMQR gene (93.7%) while, qnrC was positive in 18.7% of isolates. None were positive for gnrA and gnrS. Two (0.1%) of the isolates were positive for aac(6')-lb gene. The qepA gene regulating the efflux pump was negative in all the isolates studied. One isolate that was susceptible to all antibiotics tested negative for all the genes.

Conclusion: The emergence of Shigella sonnei with a characteristic sulphonamide resistance needs to be addressed further in detail and the increasing trend of resistance to quinolones is a point of concern. This study also shows the emergence of a particular ERIC type in the background of this evolving resistance pattern.

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Carriage of multiple gene cassettes mediated extended spectrum cephalosporinase within diverse incompatibility (Inc) plasmid groups among gram negative rods in a tertiary referral hospital of India



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**Background**: Extended spectrum β-lactamases pose to be major health problem in hospital settings worldwide. Infection with ESBL producing organisms result in poor clinical outcome, overdue initiation of suitable antibiotic treatment, longer hospital stays and greater hospital operating cost. Management of treatment against these strains become complicated when the resistant determinants are associated with integron and horizontally transferable due to their location within plasmid. In this study, we report multiple gene cassettes mediated extended spectrum cephalosporinase within diverse Inc plasmid groups among gram negative rods for the first time in India.

Methods & Materials: A total number of 458 clinical isolates of gram negative rods were collected during November 2011 to October 2013 from Silchar Medical College and Hospital. ESBL status was detected by phenotypic screening as per CLSI criteria and multiplex PCR assay followed by sequencing. Genetic environment was determined by integrase gene PCR and location of bla<sub>ESBLs</sub> within gene cassette was investigated by 59base element PCR and sequencing. Plasmid transferability was done by transformation and conjugation while incompatibility profiling was done by PCR based replicon typing. DNA finger printing of isolates was done by ERIC and REP PCR.

**Results**: A total of 56 isolates were found harboring *bla*<sub>ESBLs</sub> by PCR and sequencing. All of them were carrying class I integron and bla<sub>ESBLs</sub> was found to be located within gene cassette and conjugative plasmid. Further, PCR based replicon typing established presence of diverse Inc plasmid types viz. FIA, FIB, P, F<sub>rep</sub>B, K, B/O, 11/Iy and Y. The isolates showed high MICs against cephalosporins  $(\geq 256 \,\mu g/ml)$ , and monobactam  $(\geq 256 \,\mu g/ml)$  but was found in susceptible range against Ertapenem drug. The isolates were found clonally unrelated.

Conclusion: The study revealed presence of gene cassette mediated bla<sub>ESBLs</sub> among gram negative rods within hospital environment. Presence of bla<sub>ESBLs</sub> in diverse Inc plasmid groups suggests their diverse source of acquisition. Current study insists vital