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Molecular serogrouping of serologically atypical shigella isolates from South India

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Background: *Shigellae* are the causative agents of bacillary dysentery, represents a significant public health problem worldwide especially in developing countries. New serotypes or subserotypes in *Shigella* are not uncommon and are reported from different parts of the world. Notably, atypical *Shigella* exhibit greater antibiotic resistance than typical *Shigella* serotypes and also found to harbour integrons which has the ability to acquire resistance genes. The purpose of this work was to amplify *rfb* regions (contains the genes coding for the enzymes responsible for O-antigen synthesis) of non-agglutinating *Shigella* isolates to characterize these by endonuclease restriction and to study the presence of antimicrobial resistance genes (AMR).

Methods & Materials: A total of 3647 faeces specimens were processed between January to December 2014, among these, 27.5% (n = 176) were identified as *Shigella spp.* Eight non-agglutinable *Shigella* isolates obtained were included in the study for molecular characterization. These strains were tested for their antimicrobial susceptibility against six antibiotics by Kirby Bauer disc diffusion method. All the isolates were screened for the AMR genes (*dhfr1A*, *sullI*, *bla-OXA*, *bla-TEM*, *bla-CTX-M*, AmpC and *qnrA,B,S*) by PCR. The isolates were also amplified for their O-antigen gene cluster using Expand long template PCR system and the amplicons were further restricted using *MbolI* enzyme.

Results: The prevalence of shigellosis during the study period was 4.8%. The antimicrobial resistance for the atypical *Shigella* were 50% for ampicillin, co-trimoxazole (87%), nalidixic acid (37%), cefixime (12.5%) and all were susceptible to norfloxacin and cefotaxime. The AMR gene PCR results showed 25% of *dhfr1A* (n = 2), 62.5% *sullI* (n = 5), 50% *bla-TEM* (n = 4), 50% *qnrS* (n = 4) and all the isolates were negative for *bla-OXA*, *bla-CTX-M* and AmpC genes. *rfb*-RFLP results showed clearly identifiable and reproducible pattern, six different patterns were obtained.

Conclusion: This study revealed the description of O-specific patterns allowing serotype identification without the use of antisera. Although the number of atypical *Shigella* strains in this study was only eight, thorough and strict monitoring of isolation of such atypical strains would help to understand the actual disease burden caused by the new *Shigella* serovars and consequently to study the epidemiology of shigellosis.

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Profile of genes coding for Carbapenemases among resistant acinetobacter species from a tertiary care centre: A laboratory based study

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Background: Acinetobacter species are being increasingly reported to be associated with infections among seriously ill individuals. These infections are difficult to treat due presence of genes coding for resistance to most of the available antibiotics. The study was conducted to identify the presence of commonly described genes coding for carbapenemases among the isolates.

Methods & Materials: The study was a cross-sectional laboratory based observational study from a tertiary care hospital. 89 consecutive (single isolate per patient) isolates of Acinetobacter species were included. Study period: isolates collected during 2011-2012 from clinical samples and molecular studies done in 2013. Organisms identified as Acinetobacter baumannii complex (Acb complex) using biochemical tests were included in the study. Antibiotic susceptibility test was done by Kirby Bauer Disc diffusion. Resistance to carbapenems was confirmed by Microbroth dilution. PCR was done to identify the presence of the following genes using published primers, Oxa(23like,24like,58,51like,NDM,VIM and IMP). ERIC PCR was done to identify if there was any clonal similarity among these isolates.

Results: The isolates demonstrated resistance to nearly all antibiotics tested except for Netilmicin for which 11%(10/89) were susceptible.

The samples from which the Acb complex were isolated: Respiratory secretions (n = 60,67%), pus samples (n = 25,28%), urine (n = 10,11%) blood 2 and other fluids 3 isolates.

53,(60%) of the samples were from patients admitted to the intensive care unit.

The common mechanisms of Resistance for carbapenems were found to belong to the OXA carbapenemases(23) in 92% of isolates, followed by VIM(65%). Around 82% also had AmpC beta-lactamases. NDM detected in 12.

ERIC PCR: Isolates showing >90% of similarity were grouped into 4 different clusters, genetic heterogeneity was noticed among each cluster.

Conclusion: Acinetobacter species though previously thought to be contaminants have now gained notoriety as multidrug resistant, hospital acquired pathogens due to acquisition of a number of resistance genes. Genes coding for Carbapenem resistance identified commonly in our study were the OXA 23like and VIM. NDM was also detected. Considerable heterogeneity was noted by ERIC PCR.

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