Abstract

Title: Prevalence and Characterization of carbapenem resistant organisms causing urinary tract infections among hospitalized patients and outcomes of these infections in a tertiary care center.

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Introduction: Carbapenems have been our trusted allies in the battle against multi-drug resistant infections caused by *Enterobacteriaceae*. However, carbapenem resistant *Enterobacteriaceae* (CRE) have emerged rapidly over the past decade. Carbapenemase production is the commonest mechanism of resistance among CRE. The treatment of infections caused by CRE is a challenge because of the limited effective drug options. The current opinion is to revive the older antibiotics as treatment options against CRE.

Aim: To characterize carbapenem resistant organisms causing urinary tract infections in hospitalized patients using phenotypic and molecular methods.

Objectives:

i To determine the prevalence of carbapenem resistant *Enterobacteriaceae* causing UTI among hospitalized patients.

ii To detect the various carbapenem resistance mechanisms by performing the following tests:
   a. Phenotypic differentiation of carbapenemase and non-carbapenemase mediated mechanisms by the Modified Hodge Test, CarbaNP test and SUPERCARBA CHROMagar.
   b. Molecular characterization of $bla_{KPC,}$ bla_{NDM}, bla_{IMP}, bla_{VIM} and bla_{OXA48} like genes by multiplex-PCR.

iii. To determine in-vitro activity of colistin, fosfomycin and nitrofurantoin against the isolates and screen resistant isolates for plasmid mediated resistance mechanisms.

iv. To study the treatment outcomes of these infections

Materials and methods:

A total of 3833 consecutive urine specimens were included for calculation of prevalence of CRE UTI. And, 150 consecutive, non-repeat, specimens satisfying inclusion/exclusion criteria were included: *E.coli* (n=81) and *Klebsiella* spp (n=69) and characterized, based on gender, age, admitting department, method of sample collection, presence of co-morbidities or risk

factors predisposing to UTI and treatment outcomes in patients. *Klebsiella* spp (n=69) were reclassified into *K. pneumoniae* (n=47) and *Raoultella* spp (n=22) using latest classification scheme. Further characterization of CRE was done using Multiplex PCR for presence of *bla*KPC, *bla*NDM, *bla*IMP ,

*bla*VIM and *bla*OXA48 like genes and using phenotypic tests MHT, CarbaNP and SUPERCARBA chromagar for carbapenemase production and comparison between them.

Antimicrobial susceptibility testing using routinely tested drugs as well as older drugs of renewed interest. Susceptibilities to fosfomycin and nitrofurantoin were determined by MIC E-test. Colistin susceptibility was determined using micro broth dilution method (current gold standard). Screening of resistant isolates for plasmid mediated resistance mechanisms; Colistin- Screening of resistant isolates (n=14) using PolymyxinNP test and PCR for plasmid borne mobile colistin resistance gene mcr-1; Nitrofurantoin- Resistant or intermediate E.coli (n=36) for presence of plasmid borne *oqx*AB genes encoding efflux pump and Fosfomycin:- Isolate testing intermediate susceptible (n=1) for plasmid borne fosA3 gene

Results: Prevalence of CRE UTI was 2.9% during the study period. The 150 isolates were identified as 81 *E.coli*, 47 *Klebsiella pneumoniae* and 22 *Raoultella* species. Carbapenemase encoding genes were detected in 108 isolates. The commonest gene being *bla*NDM followed by *bla*OXA-48 like. Commonest gene in *E.coli* being *bla*NDM; in *K.pneumoniae* and *Raoultella* spp being *bla*OXA-48 like In-vitro susceptibility rates to colistin, fosfomycin and nitrofurantoin, respectively, were 95%, 100% and 75% for *E.coli* and 89%, 95% and 0% for *Klebsiella pneumoniae*; and 77%, 95% and 0% for *Raoultella* species. Colistin resistant isolates tested positive by PolymyxinNP but molecular screening for plasmid-borne mobile colistin resistance gene (*mcr*-1) was negative. Plasmid borne resistance genes *oqx*AB were absent in nitrofurantoin resistant *E.coli*.

Discussion and conclusion: The characterization of resistance mechanisms in CRE plays a vital role in hospital infection control as the carbapenemase producing genes are borne on plasmids and tend to spread rapidly in a hospital setting. SUPERCARBA CHROMagar emerged as sensitive test for carbapenemase detection including OXA-48 like producers. We found considerable in vitro activity of colistin and fosfomycin against CRE and of nitrofurantoin against carbapenem resistant *E.coli*. In a situation where most newer drugs have fallen prey to bacterial resistance mechanisms, revival of older drugs for treatment of CRE UTI may be worthwhile. Absence of plasmid borne resistance genes in organisms resistant to older drugs is encouraging.

Keywords: carbapenem resistant organisms, SUPERCARBA CHROMagar, colistin, fosfomycin, nitrofurantoin.