

Broad Spectrum Antibiotics and Resistance in Non-target Bacteria: An Example from Tetracycline

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Bacteria are posing a huge threat to human race by becoming resistant to antibiotics. Tetracyclines are broad spectrum antibiotics extensively used for treating variety of human and animal diseases and as growth promoter in animal husbandry. *Escherichia coli* is not the primary target of tetracycline but their overuse has created an overwhelming level of resistance in *E. coli*. In this experiment clinical isolates of *E. coli* (n=157) were collected from a tertiary care hospital in Pakistan and were checked for their resistance pattern to tetracycline by modified Kirby-Bauer disc diffusion methods. Significant resistance (93%) was observed among the tested isolates. This substantial resistance indicates the widespread misuse of tetracycline, putting strong selection pressure on *Escherichia coli* to evolve resistance.

Key words: Antibiotics, Tetracycline, *Escherichia coli*, MDR

Bacteria are posing a huge threat to human race by becoming resistant to antibiotics. Since antibiotics are the only effective and cheaper way to control bacterial infections and this widespread resistance is extremely dangerous. Bacteria have been found resistant to variety of antimicrobial agents which were once very effective. The disappointing aspect of antibiotics is, once bacteria are exposed, they try to withstand the antibiotics inhibitory pressure, many of them die but some of them mutate and survive. Those resistant genotypes then multiply and produce a generation of resistant bacteria, which often spread this resistance to other bacteria through horizontal gene transfer. The overuse of antibiotics speed up

the process of horizontal gene transfer among bacterial communities (Zhu *et al*, 2012), which in turn results in a complex resistant phenotype (Gillings, 2012).

Among the many readily available and cheaper antibiotics which bacteria have been found resistant to them are tetracyclines. Tetracyclines are the first broad spectrum antibiotics (Roberts, 1997), discovered in 1945 (Duggar, 1948). Tetracyclines have been used in variety of bacterial infections and have also been found very effective against other pathogenic microorganisms like chlamydia, mycoplasma and rickettsia (Michalova *et al*, 2004; Chopra *et al*, 1992). Besides their wide range applications in human medicine, they have also been extensively used in agriculture (Lau *et al*, 2008), animal husbandry and aquaculture (Daghrir and Drogui, 2013; Goossens *et al*, 2005; Miranda *et al*, 2003). Tetracycline antibiotics block synthesis of bacterial proteins by binding to the

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30S subunit of ribosome hence preventing the binding of charged transfer RNA responsible for polypeptide chain synthesis. Bacteria pose resistance to tetracyclines by any of these four mechanisms; target site modification, through ribosomal protection proteins, enzymatic inhibition of tetracyclines and efflux of tetracycline out of bacterial cells (Tuckman *et al*, 2007; Chopra and Roberts, 2001).

Escherichia coli is a commensal bacterium generally lives neutral in the intestinal tract in variety of animals and human, however sometimes it becomes virulent and causes fatal diseases (Jafari *et al*, 2012). Pathogenic strains when pose resistance to antibiotics result in severe complications. *Escherichia coli* has shown resistance to many commonly used antibiotics. Tetracyclines antibiotics are not generally administered for *E. coli* infections but still there is an overwhelming resistance found in *E. coli* against Tetracyclines (Karami *et al*, 2006). Tetracyclines antibiotics are commonly administered for skin and throat infection in Pakistan (Ullah *et al*, 2012).

The most frequent mechanism of resistance reported in *E. coli* against tetracycline is via efflux pumps (Chopra and Roberts, 2001). There are six groups of such Efflux pumps (Tuckman *et al*, 2007). And 38 genes have been reported to make these pumps (Roberts, 2005). These resistance genes spread to other bacteria through mobile genetics elements such as integrons, plasmids and transposons, (Braoudaki and Hilton, 2004). The spread of such genes in *E. coli* was first time reported in 1960 (Tuckman *et al*, 2007).

Due to this spread of tetracycline resistance determinants in *E. coli* since seven decades, an overwhelming population of *E. coli* living in different habitats has been found resistant to tetracyclines. In this experiment the clinical isolates of *E. coli* causing variety of infections were checked for their resistance pattern against tetracycline.

MATERIALS AND METHODS

Sampling and identification

Bacterial samples were collected from indoor and outdoor patients with different infection visiting Railway General Hospital, a tertiary care

hospital in Rawalpindi, Pakistan, from March 2012- March 2013. *Escherichia coli* identification was done through standard biochemical tests i.e; fermentation of lactose, Triple Sugar Ion (TSI) test, capability to produce indole, hemolysis on blood agar, utilization of citrate as described by (Cheesbrough, 2006).

Antibiotic susceptibility tests

Escherichia coli strains were cultured in Mueller-Hinton Broth and culture was adjusted to 0.5 McFarland standards. *E. coli* strains were then swabbed on Mueller- Hinton agar plates. Tetracycline discs (30ug) were placed on the plates and incubated overnight. Interpretation was done according to the recommendations of (CLSI, 2013).

Statistical analysis

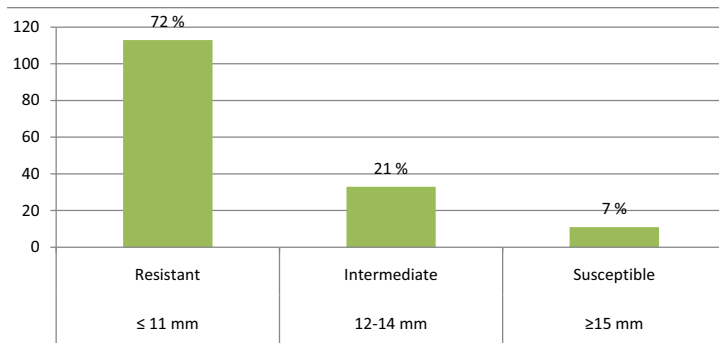
Susceptibility tests were performed in triplicate and one way ANOVA was performed to check for significant level of resistance at 5% p-value ($p \leq 0.05$).

RESULTS

Total of 157 *Escherichia coli* isolates were screened against tetracycline antibiotics. Of them 133 showed resistance, 13 were intermediate resistant and only 11 of them were susceptible Fig.1. These isolates were collected from different infection sources like urine (47%), pus (19%), wound swab (11%), HVS (8%), catheter tip (5%), blood (6%) and sputum (4%). All of *Escherichia coli* isolates from different infections showed considerable resistance Fig.2. Among the 73 isolates from urine, 64 were resistant to tetracycline and 9 were found susceptible. In the 30 pus samples, 28 were resistant and only 2 were susceptible. All of the isolates from the wound swabs, HVS (High Vaginal Swab), Catheter tip, sputum and blood were resistant to tetracycline.

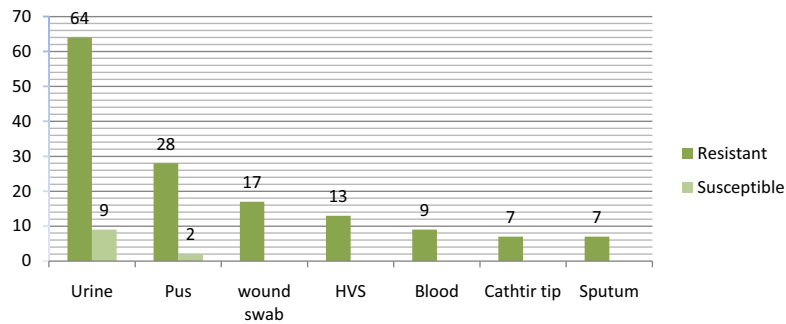
DISCUSSION

Our results of tetracycline resistance by pathogenic *Escherichia coli* strains causing extra intestinal infections indicate that the pattern of resistance is widespread and very significant. We found 93 % of *E. coli* were highly resistant to tetracycline, while in another report from Pakistan (Hassan *et al*, 2011) 95% of tetracycline resistance was found in *E. coli* isolates from urinary tract



Seventy two percent of *Escherichia coli* isolates were resistant, twenty one percent were intermediate and only seven percent of all the isolates were susceptible.

Fig. 1. *E. coli* resistance pattern to tetracycline



Among 73 isolates from urine and 30 isolates from pus, only 9 and 2 were susceptible to tetracycline respectively. In rest of the sample groups all isolates were found resistant.

Fig. 2. Resistance level of tetracycline in various sample groups

infections. This resistance is significantly high as compared to the finding from diarrheagenic *E. coli* by Ahmed *et al* in 2009. In a report from India (Chatterjee *et al*, 2009) more than 50% of *E. coli* causing urinary tract infections were found resistant to Tetracycline, while 73% of tetracycline resistance was reported in extra-intestinal pathogenic *E. coli* from Bangladesh (Fakruddin *et al*, 2013). Resistance to tetracycline is a global problem and this phenomenon keeps on increasing with the passage of time. In a two year comparative analysis of the uropathogenic *E. coli* (UPEC) resistance to tetracycline from India (Kumar *et al*, 2013), 57% of resistance was reported in 2007, which increased to 78% in the same set up in 2008.

Tetracycline resistance pattern of diarrheagenic *E. coli* seems lower as compared to that of extra intestinal pathogenic *E. coli* strains. In a study from Iran 32% of diarrheagenic *E. coli*

were resistant (Momtaz *et al*, 2013), which is significantly lower than resistance of uropathogenic strains, and strains causing other than intestinal infections. 55% resistance to tetracycline was reported in another study (Karimi, 2013) in children with acute diarrhea. It was found in United States (Sayah *et al*, 2005) that tetracycline antibiotics has the most frequent resistance in the *E. coli* isolated from faeces of human, birds, cattle and pet animals. In another report from India (Sharada *et al*, 2008) *Escherichia coli* isolated from poultry lesions showed 83% resistance to tetracycline. Tetracycline resistance in *E. coli* is not only limited to human and the animals which live in close proximity with humans, but resistant isolates have also been reported from waste water (Watkinson *et al*, 2007).

Tetracyclines were long attributed as one of the oldest and most effective broad spectrum

antibiotics but their overuse made bacteria resistant. Today, even, resistance is reported in bacteria for which Tetracyclines are not administered, such as *Escherichia coli* (Karamiet al, 2006). This could be because of several reasons. Since antibiotics do not specifically inhibit the target bacteria for which they are administered, and as there are billions of bacteria live as commensal in the body, so these antibiotics target not only the pathogenic bacteria but also exert pressure on natural body flora. As a result of selection pressure, resistant mutants arise in harmless bacterial communities which then produce generations of resistant clones. These resistant offspring if become virulent at some stages of life, like *E. coli*, cause severe diseases. Secondly, the resistant determinants in bacteria often reside on genetic elements that can move across bacteria communities and share the resistance traits. Resistance to antibiotics in *Escherichia coli* is because of resistant genes located on integrons, plasmids and transposons (Carattoli, 2009; Sherley et al, 2004). Resistance to tetracyclines has been attributed through efflux pumps. Tetracycline resistant genes that have been reported from *E. coli* strains are *tet(A)*, *tet(B)*, *tet(G)*, *tet(D)*, *tet(C)* and *tet(E)* (Wilkerson et al, 2004). Despite the overwhelming resistance reported against tetracyclines, they are still used in human medicine in many countries, including the United States (Roberts, 2003). In Pakistan tetracycline is generally used for treating skin and throat infections (Ullah et al, 2012).

Since the applications of tetracyclines are so abundant, more advanced generation of tetracycline, such as tigecycline, was designed to overcome the mechanism of resistance developed by bacteria, by having a modified structure (Tuckman et al, 2007), so that it maintains the wide spectrum of inhibition against not only *E. coli* but variety of pathogens causing diseases in humans and animals.

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