E-Test Read Wrong for the metallo-B-lactamase Phenotype in Carbapenem-resistant Streptococcus Equisimilis Causing UTI in a Zoo Lioness

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Abstract

Metallo-B-lactamase (MBL) producing bacteria are one of the most dangerous emerging threats to public health professionals. In the laboratory, the production of MBL by any bacteria is determined using double disc synergy (DDS) test (one carbapenem disc and other EDTA disc) placed at a distance of 15-20 mm while testing sensitivity for other antibiotics or using MBL E-Test strips available from Biomerieux and other producers. In our observations, we found DDS more efficient and giving clear results while chances of mistakes exist in observation of epsilon zone of growth inhibition with E-test on carbapenem-resistant EDTA sensitive bacteria. Here photo-plates are shown to make it clear using a carbapenem-resistant Streptococcus equisimilis isolated from a case of urinary tract infection (UTI) in a zoo lioness. As a cause of UTI S. equisimilis has rarely been reported, that too in a lioness. Keywords: Carbapenem resistance, MBL, E-test, Double disc synergy (DDS) test, Urinary tract infection, Streptococcus equisimilis, Lioness, Zoo animal, MDR, Herbal antimicrobials, Herbal antimicrobial drug resistance. Thyme oil, Ajowan oil, Cinnamon oil, Lemongrass oil, Patchouli oil, Agarwood oil, Holy basil oil.

1. The Case

A urine sample collected from a zoo lioness submitted to Epidemiology Laboratory at IVRI, Izatnagar was examined bacteriologically. The urine sample contained ~1200 cfu of Escherichia coli/ ml of urine and more than a million cfu/ml of Streptococcus equisimilis. Both the bacteria were identified using API tests and other biochemical and growth characteristics as per Singh (2009). On testing the isolates for antibiotic sensitivity (Singh et al, 2013) E. coli isolates were resistant to EDTA, linezolid, lincomycin, penicillin, tetracycline, ciprofloxacin, ampicillin, doxycycline, nalidixic acid and erythromycin but were sensitive to azithromycin, nitrofurantoin, chloramphenicol, co-trimoxazole, tigecycline, imipenem, meropenem, ertapenem, ceftriaxone, ceftazidime, cefotaxime, cefoxitin, piperacillin, amoxicillin, aztreonam, cefepime, spectinomycin, cefpodoxime, and moxalactam. On the other hand, S. equisimilis isolates were resistant to most of the drugs mentioned here except for EDTA, tigecycline, nitrofurantoin, chloramphenicol, linezolid, novobiocin, spectinomycin, lincomycin, bacitracin, clindamycin, and vancomycin. Additionally S. equisimilis isolates were also resistant to amoxicillin sulbactam, amoxicillin-clavulanic acid, piperacillin-tazobactam, ceftazidime clavulanic acid, and cefotaxime clavulanic acid. Besides resistance to azithromycin and erythromycin, both of the macrolides had D-effect on the inhibition zone of clindamycin.

2. MBL Phenotype

The carbapenem-resistant isolates of S. equisimilis were tested from MIC of imipenem, cefotaxime, colistin and cefotaxime+clavulanic acid, and for determining MBL using E-test strips from Biomerieux Ltd. The MIC values of imipenem, cefotaxime, cefotaxime+clavulanic acid, and colistin, for all the three isolates of S. equisimilis tested were ≥256 microgram/ml, ≥16 microgram/ml, ≥1 microgram/ml and ≥256 microgram/ml, respectively. Using MBL E-test, the MIC for imipenem was the same (≥256 microgram/ml)
but on the other side of the E-strip, containing EDTA with imipenem, MIC reading was less than 1 microgram/ml. However, the shape of the zone along the E-trip was not in epsilon shape but an ellipsoid (Fig. 1).

Fig. 1: E-test for determining MIC of imipenem, cefotaxime, colistin and cefotaxime-clavulanic acid to detect MBL potential of Streptococcus equisimilis isolate from a lioness case of UTI

Fig. 2: Antibiotic sensitivity assay and Double disc synergy test for Streptococcus equisimilis isolate from a lioness case of UTI

3. Herbal Antimicrobial Drug Sensitivity of the Isolates

Both E. coli and S. equisimilis isolates were tested using disc diffusion assay (Singh et al., 2013) against essential oils (1 micro l/ disc) of ajowan, betel leaves, guggul, patchouli, thyme, agarwood, sandalwood, holy basil, lemongrass, cinnamon and pure compounds of herbal origin including cinnamaldehyde, carvacrol and citral, active antimicrobial ingredients of cinnamon oil, thyme oil (oregano and ajowan too) and lemongrass oil, respectively. Besides silver nanoparticles made using herbal acacia gum synthesis were also tested for their antimicrobial activity. The isolates of both the organisms (E. coli and S. equisimilis) were insensitive to essential oils of guggul, patchouli, agarwood, lemongrass and also to citral. However, isolates were sensitive to carvacrol, ajowan oil, thyme oil, cinnamon oil, and cinnamaldehyde. Betel leaf oil, holy basil oil and silver nanoparticle inhibited only E. coli isolates and sandalwood oil inhibited S. equisimilis isolates only.

The observations revealed that for in carbapenem-resistant but EDTA sensitive Streptococcus equisimilis E-test may give erroneous results and combined discs may also be read as MBL. To determine MBL double disc synergy (DDS) is a better test in case of EDTA sensitive strains, the observations are in contradiction to observations of Picao et al. (2008) indicating equivocality of the combined disc and DDS tests for detection of MBL. The case study also revealed that UTI infections in lions may also be associated with S. equisimilis infections. Further, the bacteria resistant to even most of the modern antimicrobials was sensitive to some of the herbal antimicrobials indicating the utility of herbal antimicrobials in the treatment of infections caused by MDR strains.

References

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