ESBL PATHOGENS IN STERILE FLUIDS

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BACKGROUND-

The ESBL producing organisms wee first detected in Germany in 1983 (* H. Knothe et al ,Infection Volume 11) mainly
include members of the family Enterobacteriaceae; examples being Escherichia Coli, Klebsiella Pneumoniae, Enterobacter species, Klebsiella ozaenae, Acinetobacter species, Citrobacter freundii, Klebsiella oxytoca , Proteus species, Providencia species.(* Bailey and Scott Diagnostic radiology 11th edition) There are many been new forms of antibiotic resistance that have
emerged including the AmpC beta lactamases, the Metallobeta lactamases and one of the most recent NDM 1 (New Delhi Metallobeta lactamase 1) which was cited in the Lancet Infectious Diseases Journal in 2010.. However owing to the the overuse and misuse of drugs by the doctors as well as the patients ,resistance against these antibiotics has emerged yet again in the form of ESBLs or the Extended spectrum beta lactamases.

AIMS AND OBJECTIVES

Detection of Extended spectrum beta lactamases from archived isolates recovered from sterile fluids 2009-2010.

MATERIALS AND METHODS

The study was performed at the Department of Microbiology, Vardhaman Mahavir Medical College and Safdarjung Hospital for a period of two months. A Total 50 archived multi drug resistant gram negative bacillary isolates from various sterile fluids were randomly selected, preserved from October 2009 to June 2010 were included in the study .These were first subcultured and then antibiotic sensitivity was performed as per CLSI 2009. Disc diffusion test was applied to screen for antibiotic resistance. Screening for ESBL was carried out using DDST (Double disc synergy test), and the results were further confirmed using E strip MIC method.

KIRBY BAUER TEST (Antibiotic disc diffusion sensitivity testing)

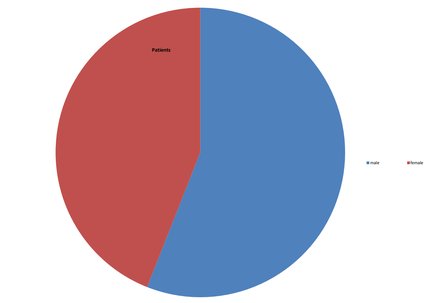
An agar plate (Mueller Hinton agar) is inoculated with the test organism and filter paper disks impregnated with antibiotics are placed on its surface. If the bacteria are susceptible to a particular antibiotic, an area of clearing surrounds the disc where bacteria are not capable of growing (called a zone of inhibition).The diameter of the inhibition zone is a function of the amount of drug in the disk and susceptibility of the microorganism. This test allow the designation of an organism as "susceptible", "intermediate", or "resistant" to concentrations of an antibiotic which can be attained in the blood or other body fluids of patients requiring chemotherapy (*laboratory detection and reporting of bacteria with Extended spectrum beta lactamases Issue no: 2.2 by: Standards Unit, Evaluations and Standards Laboratory)

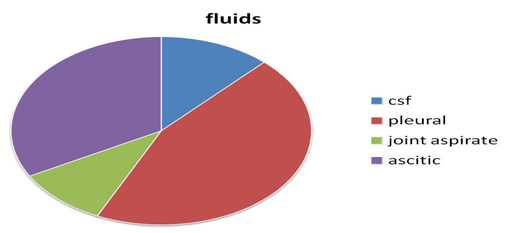
ESTRIP

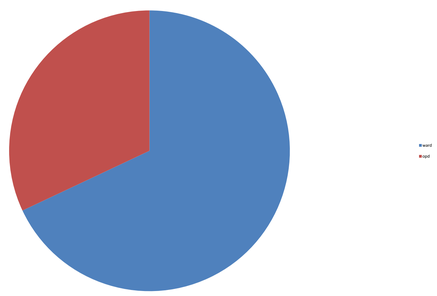
E test ESBL strips are two sided strips that contain a gradient of ceftazidime on one end (MIC test range 0.5 to 32 microg/ml) and ceftazidime plus clavulanate on the other end. (*Etest ESBL Manual) These strips are useful for both screening and phenotypic confirmation of ESBL production A positive test for an ESBL is a more than 3-dilution reduction in the MIC of ceftazidime in the presence of clavulanic acid (4 microg/ml).The E test uses the principle of agar diffusion to perform quantitative testing. In order to determine a MIC(minimum inhibitory concentration) with the E test, the surface of an agar plate is swab inoculated with an adjusted bacterial suspension in the same manner as a disk diffusion test. One or more E test strips for the antimicrobial agents to be tested are then placed on the inoculatated agar surface. After an overnight incubation, the interaction of the antimicrobial agent gradient and the test bacterial inoculum gives rise to elliptical inhibitory zones. The results are read in the intersection of the ellipse with a MIC scale on the strip.

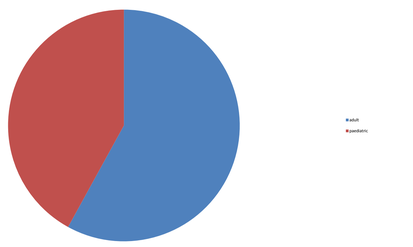
DOUBLE DISC DIFFUSION TEST

This test utilises the synergy between cefotaxime and clavulanate by placing a disk of amoxicillin/clavulanate (20 microg/ 10microg, respectively) and a disk of cefotaxime (30 microg), 30 mm apart (center to center) on an inoculated agar plate. A clear extension of the edge of the cefotaxime inhibition zone toward the disk containing clavulanate was interpreted as synergy, indicating the presence of an ESBL . current CLSI (Clinical and laboratory standards institute) recommendation (*Jan 2010 20th international supplement Vol.30,No.1) consists of determining MICs(minimum inhibitory concentration) of either ceftazidime or cefotaxime with or without the presence of clavulanic acid(inhibitor ofbetA lactamases). A decrease in the MIC of 3 fold dilutions in the presence of clavulanate is indicative of the presence of an ESBL. If an ESBL is detected, the strain should be reported as nonsusceptible to all expanded-spectrum cephalosporins

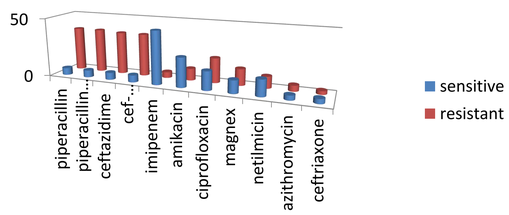




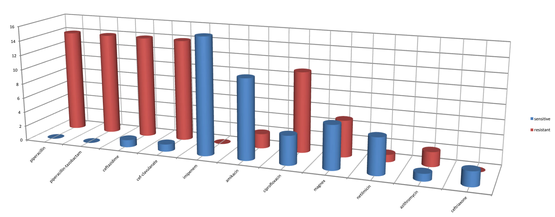




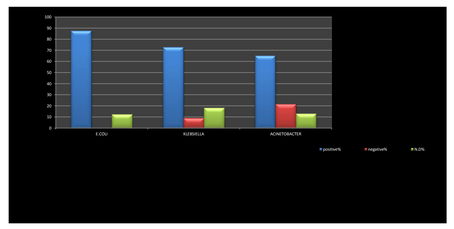
All Isolates Disc Diffusion Results



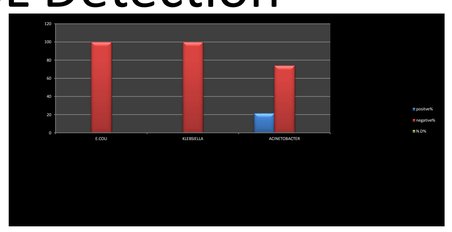
E.Coli



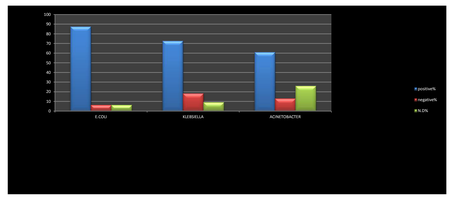
AmpC Detection



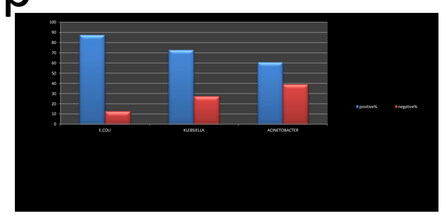
MBL Detection



ESBL Detection

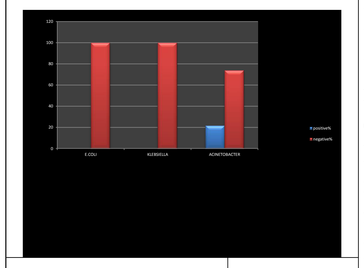


ESBL (Confirmation)E Strip

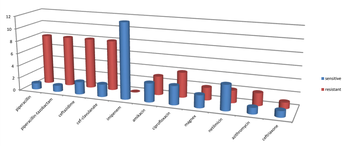


CONCLUSIONS The latest study from The Lancet(17) Infectious diseases journal in 2010 on NDM strain provides us a strong indication to use antibiotics carefully In the clinical setup and to not underestimate the power of antibiotic resistance And the harm that it can do to mankind. Our study too elucidates how widely prevalent the ESBL producing bacteria are; which are now being recovered even from more serious infections including CNS infections and also that MBL resistance is the current budding threat to mankind.

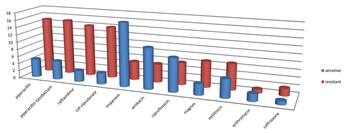
MBLConfirmation(E Strip)



Kliebsiella



Acinetobacter



Discussion

ESBL producing organisms are among the fastest growing problems in the area of infectious disease. Clinical microbiological laboratory can no longer rely on simple in-vitro susceptibility in the absence of proper detection of ESBL. ESBL isolates were first detected in Western Europe in the mid 1980s. These, with the increasing incidences, are able to hydrolyze 3rd and 4th generation cephalosporins, monbactams. Beta-lactamase inhibitors (clavulanic acid, sulbactam, tazobactam) inhibit ESBL producing strains. (4) & (5).Beta-lactam antibiotics are a broad class of antibiotics with a heteroatomic ring structure. The Beta-lactam ring is a part of the structure of several antibiotic families principally the penicillins, cephalosporins, carbapenems, monobactams, therefore, a.k.a beta-lactam antibiotics. Resistance to these antibiotics emerged in the form of beta-lactamases. The beta-lactamases break the 4-membered beta-lactam ring thus inactivating it. The integrity of beta-lactam ring is necessary for the activity which results in inactivation of a set of transpeptidases that catalyze the final cross-linking reactions of peptidoglycan synthesis. ESBL producing organisms often also able to decrease the susceptibility of other non-beta-lactamses antimicrobial classes such as aminoglycosides, fluoroquinolones, TMP-SMX, tetracyclins and nitrafurantoin (6). The prevelance of ESBL among pathogenic bacteria varies geographically and in hospital settings and is rapidly changing over time (7). The frequency of ESBL in Europe is higher as compared to that of the USA but lower than Asia and South America. (8) and (9).The ESBL producing organisms mainly include members of the family Enterobacteriaceae; examples being Escherichia coli, Klebsiella pneumoniae, Enterobacter species, Klebsiella ozaenae, Acinobacter species, Citrobacter freundii, Klebsiella oxytoca, Proteus species, Providencia species. Also, ESBL resistance has been reported in Pseudomanas aueroginosa. Presence of ESBL producing bacteria ranges from 23-86% in India. The ESBL producing bacteria have been implicated in several infections including CNS infections like meningitis in neonates and adults, subdural empyema, brain abscess, septicemia and neonatal sepsis. Recent studies revealed that patients with infection such as septicaemia with ESBL producing organisms had siginificantly higher fatality rates than those with non-ESBL isolates. (10).The two most common plasmid mediated beta-lactamse are TEM-1 and SHV-1 family that confer resistant to antimicrobial mainly E.coli and K. pneumoniae respectively. (11).Detection of ESBL has become a major challenge for the clinical laboratory. (12).Once ESBL is suspected, it should be confirmed by standardized methods. (13). Several methods have been developed to detect ESBL including Double-disk synergy test (DDST) and Double-disk diffusion test (DDDT) using cefotaxime and ceftazidime disks with or without clavulanic acid. (14).

RESULTS The maximum samples recovered belonged to cinetobacter (46%). The maximum isolates show resistance to Piperacillin and piperacillin-tazobactam , followed by efazidime and ceftazidime-clavulanate. The least resistance occurs to imipenem. Total 72 % of the isolates are positive for the presence of ESBL 12% are negative and 16% are not determinable. The highest ESBL producers are recorded in E.coli (87.5%) ,followed by Klebsiella (72.72%) and then by Acinetobacter (60.86%). (P value < 0.05) Total 74% of the isolates test positive for AmpC and 12% test negative and 14% of the results cannot be determined. MBL production is recorded only by Acinetobacter species (21.73%). E strip method has been used to confirm the presence of ESBL . By this method 72% of the total isolates test positive and 28% of them test negative for ESBL production. The highest ESBL production has been recorded in E. coli (87.5%) ,followed by Klebsiella (72.72%) and then by Acinetobacter (60.85%). (P value < 0.05). MBL detection has also been confirmed by E strip method .10% of the total isolates record MBL production and 90% of them are non MBL producers.Out of the 3 species, only Acinetobacter is an MBL producer (21.73%). 100% of the E. coli and Klebsiella isolates are non MBL producers and 73.91% of the Acinetobacter isolates (P value < 0.05)

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