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Colistin Susceptibility Testing Of Multi - DrugResistantKlebsiellaPneumoniaeByBrothDisk Elution Method.

Bimba KH, Suhani Manasa*,Umesh T,Bhavana J ,Mahantesh S
corresponding author

Abstract
Background:- The purpose of this study is to explore the diagnostic utility of colistin broth disk elution (CBDE) as a simple and reliable method of colistin susceptibility testing .**OBJECTIVE:-** This study was conducted to determine the colistin susceptibility testing of MDR Klebsiella pneumoniae by CBDE method in our pediatric super-speciality hospital.**Introduction:-** Colistin (polymyxin E) is a polymyxin antibiotic that is prescribed as an agent of last resort for the treatment of multidrug-resistant (MDR) Gram-negative bacterial infections, increasing colistin resistance is crucial to report colistin MIC with a validated method, so we would like to recommend CBDE test for routine MIC reporting of colistin since it is feasible test and essential for all clinical diagnostic laboratory. Helps the clinicians to use the drug efficiently for treatment purposes.**Method:-89** MDR Klebsiella isolates were tested for colistin MIC by CBDE method as per CLSI guidelines, performed with four 10-ml cation-adjusted MHB tubes per isolate , to which 0, 1, 2, and 4 colistin 10-µg/ml disks were added, generating final concentrations in the tubes of 0 (growth control), 1, 2, and 4 gg/ml, respectively. MICs were evaluated visually and interpreted using CLSI breakpoints.**Results:-** In this present study of 89 MDR 71(82%) isolates were susceptible showing MIC of <1 gg/m1,10(11%)isolates showed MIC of< 2µg/ml interpreted as intermediate susceptible, and 6 (7%)isolates shows A gg/m1 interpreted as resistant.**Conclusions:-** With the increasing colistin resistance, it should be a mandate scope of every Microbiology laboratory to report colistin MIC by validated method, so that clinicians will use this high-end drug with caution. We hereby conclude that CBDE test is feasible and can be implemented as a part of routine antimicrobial susceptibility testing of colistin.

Keywords: *multidrug-resistant,colistinbrothdiskelution*

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I. Introduction

Over the last two decades, clinical interest in polymyxins has increased due to theemergenceofextensivelydrug-resistantgram-negativebacteriacoupledwiththedryantibiotic development pipeline. Colistin and pmb are currently considered a last-linedefenseagainsttheproblematicgram-negativeorganism's,notablycarbapenem-resistantenterobacteriaceaeand(cre)especiallyklebsiellapneumoniae[1].

Klebsiella:-

Thetribe:-klebsielleaeincludesfourmajorgenera

Klebsiella,enterobacter,hafnia,andserratia,eachofwhichincludesseveralspeciesthatareovert andopportunisticpathogensinhumans.

Klebsiella species are usually found as commensals in human intestines and assaprophytesinsoil.Non-motileandcapsulated(possesscapsularpolysaccharide).Thegenusincludessixspecies,

Klebsiellaspeciesare:-

- K.Pneumoniae,
- K.Oxytoca,
- K.Granulomatis,
- K.Variicola,
- K.Singaporensis,
- K.Alba.

Itis responsibleforpneumonia,urinarytractinfections,meningitis(neonates),septicemia,Pyogenicinfections

suchasabscessesandwoundinfections,itfrequently colonizesthe oropharynx of hospitalized patients and is a common cause of nosocomialinfections,somestrainscanrarelycauseDiarrhoea.Mostofthehospitalstrainsaremultidrugresistant.Mdrwasdefinedasacquired non-susceptibility to at least one agent in three or more antimicrobialcategories, xdr was defined as non-susceptibility to at least one agent in all buttwoorfewerantimicrobialcategories(bacterialisolatesremainSusceptibletoonlyoneortwocategories) andpdrwasdefinedasnon-susceptibilityToallagentsinallantimicrobialcategories14.

Detectionofcolistinresistanceisachallengeinclinicaldiagnosis,andevolvingbreakpointshavecomplicatedtheproblem16.

Importanceof cbde:-

- □ Theclinicaluseofcolistinhasbeenfraughtwithchallengesrelatedtodrugtoxicity,Limitedpharmacokinetic-pharmacodynamicdata,thelackofrobustclinicaloutcomesStudies,andissueswith antimicrobialsusceptibilitytesting(ast)ofthedrug17.

TheIssuessurroundingasttestingare2-fold:

(i) Thephysiochemicalpropertiesofthedrug,whichrenderinvitroastchallenging. Due to insufficient data to establish colistin clinicalbreakpointsforenterobacteriaceae,

(ii) Epidemiologicalcutoffvalues(ecvs)forcertainenterobacteriaceaewereestablished by the clinical and laboratory standards institute (clsi) basedon mic distribution data 18. Ecvs allow for the differentiation betweenisolates that have mics above the wild-type distribution (i.e., those withacquiredand/ormutationalmechanismsofresistancetocolistin)andThosethathavemicswithinthewild-typedistribution.AscolistinisAlarge,positivelychargedmolecule,itdiffusespoorlythroughagarbasedmediaandadsorbstonegativelychargedplastics,suchasPipettetipsandpolystyrenetubesandplates19.

- □ Ast of colistin by broth microdilution (bmd)was strongly recommended byclinical and laboratory standards institute (clsi), but since 2020, clsi hasalsoapproved colistin brothdiskelution(cbde)andcolistinagartest(cat)20.Bmd was could not be implemented routinely, because of labor-intensiveprocedure. Whereas, both cbde and cat can be implemented for testingcolistin susceptibility for clinical diagnostics, as it is comparatively easy toperform.
- □ Itis essentialforallclinicaldiagnosticlaboratorytostandardizecolistintestingmethodconsideringtheresourcesavailableandthisinturnwillaidcliniciansTousethedrugefficientlyfortreatmentpurposes.Inviewofthis,studywasundertakentoevaluatetheresultsofcbde21.
- □ Theaimofthestudyistodeterminethecolistinsusceptibilitytestingofmultidrugresistantklebsiellapneumoniaebybrothdiskelutionmethod(cbde)inapediatricsuper-specialtyhospital.

II. MaterialsAndMethods

An observational study was conducted in microbiology laboratory, tertiary carehospital, bangalore, south india. Sample collection was done from august to july2023. During this period, about 89 clinical isolates derived from routine clinicalsamples,suchasblood,urine,stool,pus,endotrachealaspirate,sputum,sterile bodyfluids (bile, ascitic fluid, csf) and other exudate specimens, that were showinggrowth of klebsiella pneumonia, on testing found to be multi drug resistant wereincluded in the study.as there was no intervention involved, informed consent wasnot taken from the patients. All 89 study clinical isolates were subjected to colistinbrothdiskelution(cbde)method.

Gramstaining:klebsiellaisshort,plump,straightgram-negativerod.Capsulatedandabout1-2µmx0.5-0.8µminsize2.

Bacterialstrains:-

All the routine clinical samples which were sent to microbiology lab for the cultureandsusceptibilityareprocessed,onmacconkeyagarandbloodagar,andincubatedfor 24 - 48 hrs. At 370 c and identified as per routine biochemical tests like citrateutilization test, urease test, triple sugar iron test, and peptone water , mannitolmotility

Testalongwiththeknowncontrolstainforqualitycontrol.And/orvitek-2,culture: on macconkey agar, it produces large dome shaped mucoid (due tocapsule)sticky,pinkcolor,lactose fermentingcolonies2.

Klebsiellapneumoniaeidentifiedbythebiochemicalreactions.

- Indoletest:negative
- Citratetest:positive(citrateisutilized)
- Ureasetest:positive(ureais hydrolyzed)

- Tsi(triplesugarironagartest):showsacid/acid,gaspresent,h2sabsent
- Sugarfermentationtestfermentsmostofthesugarssuchasglucose,lactose,mannitol,maltose(butnotsucrose),withproductionofacidandgas
- Mr (methyl red) test: negative ,vp(voges-proskauer)test:positive

III. Results

Inthedurationof11monthsfromaugust2022tojuly2023,12,535clinicalsamplesuch as urine ,blood, pus, body fluid, exudate samples which were received in ourlab

Fortheculture and susceptibility testing, among this 2,764 were positive for bacterial Growth shown in the chart 1 . In culture positive pathogens 504 were klebsiellaisolates(470wereklebsiellapneumoniaeand34isolatesareklebsiellaoxytoca)showninthechart1.

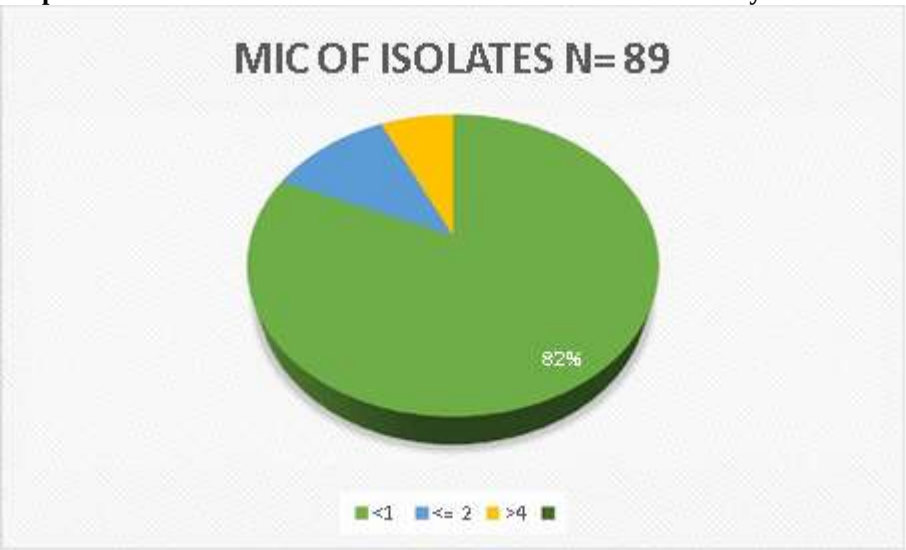
In504isolatesonperformingantibioticsusceptibilitytesting,weidentified325isolates as mdr, 247 as possible xdr, and 99 isolates were identified to bepossiblePdr.Showninthechart3.

89 mdr klebsiella pneumoniae isolates collected from consecutive samples wereincludedfor thisstudy.Mdrklebsiellaisolateswereprocessedforcbdemethodto

Checkformicofcolistin .Cbdewasperformed with four10-ml cation-adjustedmueller-hinton broth tubes per isolate, to which 0, 1, 2, and 4 colistin 10-µg/mldisks

Were added, generating final concentrations in the tubes of 0 (growth control), 1, 2,and 4 µg/ml, respectively. Mics were evaluated visually and interpreted usingclinical and laboratory standards institute breakpoints. Out of 89 mdr 73 isolateswere susceptible showing mic of <1 µg/ml , 10 isolates showed mic of < 2 µg/mlinterpreted as intermediate susceptible, and 6 isolates shows >4 µg/ml interpreted asresistant,showninthetable4.amongthese6resistantisolates4wereurinesamplesfrom drug resistant uti cases ,1 blood sample from meningitis case , 1 pus samplefromanonhealingwound,

Graph4:89clinicalisolatesofmdrklebsiellawereshownmicofcolistinbycbdemethod.



IV. Discussion

We performed this study, identifying the cbde method as a practical approach forclinical laboratories to perform colistin ast for klebsiella pneumonia in a resourcelimited setup. Cbde method was performed using readily available and affordablesupplies. Cbde applies the same principle that was used to determine anaerobeantimicrobialsusceptibility,whereantimicrobialdisksofa10µg concentrationwereeluted in a set volume of broth, to obtain standard doubling dilutions to determinemics. Clsi and eucast currently recommend that colistin ast was performedby bmd, a method few laboratories have access to . Some laboratories may chooseto perform laboratory intensive and expensive approaches, such as agar dilution andautomation, but these require significant technologist time for test preparation andsetupandarenotcurrentlyrecommendedbyclsiandeucastfor colistintesting. Furthermore, despite the advice of standards-setting organizations(clsi/eucast) and the high error rates reported for disk and gradient diffusionmethods even in automated ast method, some laboratories continue to use theseapproachesduetothelowcostandavailabilityofnecessarymaterials.

Alternatively, labs may decide to outsource colistin mic testing, but prolongedturnaround times can lead to substantial delays in the time to optimal therapy forcritically ill patients. Cbde overcomes many of the barriers that exist with theavailable approaches for colistin mic testing and offers a practical, albeit

stillaccurateandreproducible,alternativeforcolistintestingforlaboratoriesofallsizes.

Thetimelyandaccurateresultsofcolistin susceptibilityfromlaboratoriestoacriticalpatientsuchasinicucanbelikesavingmanyatimes.

Cbde method was performed for 89 mdr klebsiella isolates to check the mic ofcolistin, and it was noted that 7% isolates were colistin resistant and proving thatcolistin was still an option for treatment in 83 of the above cases, where as in theremaining 6 cases clinicians will have to consider other combination therapy oralternative expensive medications for the patients. Studies done by various otherauthors also shows similar resistance pattern, an indian study by arjune r et al.24colistin-resistant isolates were reported over 18 months . Colistin mic was >16mcg/ml in 14 isolates (58.33%), 8–16 mcg/ml in 3 isolates (12.50%), and 4–8mcg/ml in 7 (29.17%) isolates22.ramesh n et al, colistin resistance among gram-negative isolates, with colistin mic assessed by agar dilution method and withoutclinicaldata,showedthatamong94mdrisolates,[27]showedcolistinresistanceandamong9k.[29]pneumoniaeisolates6werecolistinresistant[32].Sujathasretal

,referencebmdwasperformed for 100 study isolates of cre, and it was noted that11% isolates werecolistin resistant [20]. Sarumathi d et al. Reported 20.4% colistinresistanceincreisolates36. Reported36.1%ofcarbapenemresistant k.Pneumoniaewerecolistinresistant37.basedontheresultsofamulti-centricstudybysimneretal,cbdewasprovisionallyapprovedfortestingofenterobacteralesand

P. Aeruginosa by the clsi antimicrobial susceptibility testing (ast) subcommitteein2019.Overall,94.4%and97.9%ofcbderesultswereinessentialan categoricalagreement,respectively,withrbmdmics[35]**.**

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