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Developmentof*in-vitro*SensitivityTestingforPathogenicBacteria

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Abstract
Anewmethoddevelopedfor*in-vitro*susceptibilitytestinmedicallaboratoriesconsistofmicrotubesorglovescontainingdehydratedtrypticsoyabroth,5%glucose,0.1%bromothymolblueandonetypeofantibiotics(ampicillin,tetracyclineandchloramphenicol)withcriticalconcentrationMIC(minimuminhibitoryconcentration)forsusceptibility.Standardqualitycontrol strainsofbacterial(*Escherichiacoli*,*Staphylococcusaureus*and*Pseudomonasaeruginosa*)suspensionwereadjustedto0.5McFarlandturbidity standard(1×10^6cell/mL)wereusedininoculationthemediaandincubatedtwohoursat37°C.TheMICofampicillinagainst*E.coli*,*S.aureus*,and*P.aeruginosa*were4,32,and256µg/mLofthemediaforthebacteriarespectively,whiletheMICoftetracyclineagainstbacteriawere512,512and32µg/mLrespectively,theMICofchloramphenicolwere512,32and512µg/mL,respectively.Where,theresistantbacteriatotheantibioticscouldgrowandfermentglucosesugarproducingacolorchangeofthemediafrombluetoyellow,whilethesensitivebacteriadonotgroworshownochangeincolor.Ourstudyresultcomparedwithcommonusedantibioticdiskmethodobtainingsimilarresults.Thisdevelopedmethodcharacterizedbyfast(onlytwohours)andlesscostincomparisontoconventional technique.Thenewmicrotubestripishighlystable(morethanoneyear) withmoresensitiveindetectionofvariablepathogenicbacteriaincluding standardbacteriastrainscomparedwithconventionaltechnique.

Introduction

Theclinicalsymptom ofaninfectiousdiseasereflects the interaction ofthepathogenic microorganism with the host.Thisinteractionisaffectedbymicrobialvirulencefactors and thehostimmunestatus.The symptoms and signs are differenceaccordingtothesiteandseverityofinfection[1].Thediagnosisrequiresacompositeofinformationincludinghistory,physicalexamination,radiographicfindings,andlaboratorydata[2].
Thedeterminationofmicrobialsusceptibilityto antimicrobials is very important responsibilityofthemicrobiologylaboratoryafter microbialdetectionandisolation[3, 4].Thetermsusceptiblemeansthatthemicroorganismisinhibitedbyaconcentrationofantimicrobialagentthatcanbepresentinbloodwiththenormallydependeddoseoftheantimicrobialreagentandsuggestedthataninfectionoccurredbythismicroorganismmaybeappropriatelycontrolled withtheantimicrobialagent.Microbialresistantindicatesthatthemicroorganismisresistanttoconcentrationsoftheantimicrobialagentthatcanbeobtainwithnormaldosesandimpliesthatanmicrobial infectioncouldnotbesuccessfullytreatedwiththisantimicrobialagent[5,6].
Manybacteriahaveunpredictablesusceptibilities toantimicrobialagentsandtheirsusceptibilities canbemeasured*invitro*tohelpthechoiceofthemostappropriate antimicrobialagent.Thewidelyusedsusceptibilitytestingmethodsarethediskdiffusionandbrothagardilutiontests.TheMIC(minimuminhibitory concentration)ofaparticulardrugtoaorganismcanbequantitativelydetermined*in-vitro*throughthebrothagarordilutiontest.Thesetestingmethodshavebeenstandardizedandthe NCCLS(NationalCommitteeofClinica1LaboratoryStandards)providessusceptibilitytestguidelines[6-8].Inthisstudywetriedtoimprovenewtechniquef

orsusceptibilitytestofpathogenicbacteriatothea ntibiotics.

MaterialsandMethods

MeasurementofMIC(MinimumInhibitoryConce ntration)

Stocksolution:Ampicillin(Aldrich/Sigma)(50m g/mL),Tetracycline(Aldrich/Sigma)(5mg/mL), Chloramphenicol(Aldrich/Sigma)(34mg/mL).

AgarDilution

Theanti-bacterialagentswasmeasuredbyusingbroadsp ectrumantibiotics(ampicillin,tetracyclineand chloramphenicol)ofdifferentconcentrations (0.5,2,4,8,16,32,64,128,256and512µg/mL) andinoculatedwithstandardtestedorganisms (*E.coli*,*S.aureus*and*P.aeruginosa*)whichwer epreparedbymixingpartofthegrowthfromeachof 5similarcoloniesinsalineandincubatedat37°C for2hours.

Turbidity of the suspension was adjustedto0.5McFarlandstandards(BioMerieux ,France)spectrophotometric allyat600nmwavelength(1×106cell/mL)[9-11].

PreparationofSusceptibilityStripTest

Theteststripconsistsofmicrotubescontainingdeh ydrated(lyophilized)mediatrypticsoyabroth,glu cose(5%)andbromothymolblue(0.1%)andbro ad-spectrumantibioticsincriticalconcentrationteste dforallantibioticswereequivalenttotheMICbr eakpointforsusceptibilityinsterilecondition.Th epHwasadjustedto7.4(Alkaline,bluecolor).

ApplicationofStripTest

Bacterialsuspensionofparticularmicroorganis ms(*E.coli*,*S.aureus*and*P.aeruginosa*)wereinocu latedintubescontainingvariousantimicrobialage nts.Afterincubationfor2hoursat37°C,resultswer ereported(asachangeincolorofthemedia)bynake deye.

*ConventionalAntibioticdiscsMethod*Inoculums ofthetestorganismwerepreparedasbefore.Ste rilecottonswabsweredependedinthetestandco ntrolorganismsseparately.Theseswabswere usedin inoculation of the specified areas of thePetri-dishes with testand control organisms.

Later flamed forceps used to apply Antibioticdiscswith lightpressureonthe agar surface after theinoculumshaddried.Finally, the Petri-disheswereincubatedfor18-24hoursat 37 °C andtheresultswerereported(radialwidthofthezon esoutsidetheantibioticdiscs)bynakedeye[11].

ResultsandDiscussion

Table1showdifferentconcentrationsofampicilli ntestedagainststandardbacterialsuspensionof*E. coli*,*S.aureus*and*P.aeruginosa*wereinoculate dinthemedia.Testedcriticalconcentrationsinster ileconditionforampicillinwereequivalenttoMIC breakpointwhichwere4,32,256µg/mLfortestedb acteria,respectively.While,theaveragenumber ofbacteriawere15.6×10^6,12.9×10^6and2.1×10^6b acteria/mL,respectively.The*E.coli*wasmorese nsitivetoampicillinfollowedby*S.aureus*andthen *P.aeruginosa*.

Table2showstheresultsoftestingthesensitivity ofstandardpathologicalbacteria(*E.coli*,*S.aure us*and*P.aeruginosa*)fortetracyclineusingdiffere ntconcentrationsoftheantibiotics.Whereitwas notedthatthefocusMICtothebacteriawere512,51 2,and32µg/mL,respectively,andtheaveragen umberofbacteriawere16.2×10^6,16.2×10^6,an d5.4×10^6bacteriapermL,respectively.

*P.aeruginosa*wasseentobemoresensitivetotetrac ycline,whereasthebacteria*S.aureus*and*E.coli*ha dthesamedegreeofsensitivity.

Table 1 MICs of ampicillin for different concentrations *of E. coli*, *S. aureus* and *P. aeruginosa*.

Isolates	No. of bacteria(1 × 10$_6$) for different concentration of ampicillin (µg/mL)									
	0.5	2	4	8	16	32	64	128	256	512
E. coli	34.2	33.3	15.6	32.7	29.4	6.9	26.7	25.2	24	21
S. aureus	24.3	16.8	29.4	16.2	15.9	12.9	3.3	18.9	15	21
P. aeruginosa	20.4	8.1	7.8	21.9	8.4	4.9	3.3	2.4	2.1	1.8

Table 2 MICs of tetracycline for different concentrations of *E. coli*, *S. aureus* and *P. aeruginosa*.

Isolates	No. of bacteria(1 × 10$_6$) for different concentration of tetracycline (µg/mL)									
	0.5	2	4	8	16	32	64	128	256	512
E. coli	48.3	39.9	37.5	35.1	32.4	29.1	20.1	14.4	19.2	16.2
S. aureus	20.4	17.7	22.2	33.3	9.6	25.8	20.1	18.6	17.7	16.2
P. aeruginosa	13.5	10.5	7.2	21	5.1	5.4	7.2	8.7	7.2	6.6

Table 3 MICs of chloramphenicol for different concentrations of *E. coli, S. aureus* and *P. aeruginosa*.

Isolates	No. of bacteria (1 × 10₆) for different concentration of chloramphenicol (µg/mL)									
	0.5	2	4	8	16	32	64	128	256	512
E. coli	21.9	6.6	3.3	9.9	17.1	14.4	13.2	12.3	6	4.2
S. aureus	42.3	37.8	25.8	23.7	22.8	21.9	26.4	26.1	24.3	13.8
P. aeruginosa	15.6	11.1	6.6	29.1	7.5	21.9	10.2	3.9	5.1	4.8

Table 4 MICs and MBCs of ampicillin, tetracycline and chloramphenicol among *E. coli, S. aureus* and *P. aeruginosa*.

Isolates	Ampicillin		Tetracycline		Chloramphenicol	
	MIC, µg/mL	MBC, µg/mL	MIC, µg/mL	MBC, µg/mL	MIC, µg/mL	MBC, µg/mL
E. coli	4	32	512	128	512	4
S. aureus	32	64	512	16	32	512
P. aeruginosa	256	512	32	16	512	128

Whereas,Table3showedsensitivitytestofdifferentconcentrationsofchloramphenicolagainstthestandardpathogenicstrains(*E.coli*,*S.aureus*and *P.aeruginosa*).TheMICofthebacteriawere512,32,and512µg/mL,respectively.Theaveragenumberofbacteriawere4.2×10^6,21.9×10^6and4.8 ×10^6bacteriaperml,respectively.Itwasnotethat*S .aureus*wasmoresensitivetochloramphenicol, whereasthebacteria*E.coli*and*Pseudomonasaeruginosa*hadthesamedegreeofsensitivity.
InadditiontotheMICvalue,theMBC(minimum bactericidalconcentration)valueofantibioticswasestimatedforallpathogenicstrainsasitisshowninTable4.
ThoughthevaluesoftheMBCofampicillinwere 32,64,and512µg/mLfor*E.coli*,*S.aureus*and*P. aeruginosa*,respectively,butMBCoftetracyclinewere128,16and16µg/mLforstandardbacteria,respectively.
TheMBCofchloramphenicolwere4,512,and128µg/mL,respectivelyfortestedbacteria.
Inthisstudyglucosesugarselectedinthepreparationofthenewteststripbecausemosttypesofbacteriacontainingtheenzymefermentedglucosesugar.Bromothymolbluedyewasthemostsuitabledyeusedtoindicatefermentationprocessandcannotethecolorchangeclearlywiththenakedeyesasitisin Fig.1.
Theprocessoffreezedryinghadimportantroleinmaintainingculturemediainthepocketsofteststripsandcontrolsmallquantitiesusedinadditiontotheabilitytokeepforalongtimeatlowtemperatures(4-6°C).
Weconcludedfromtheresultsofthestudyprovideagreatopportunitytoworkindiagnosticlaboratories,wherevertheyarelocatedinsideoroutsidetheprovinces.Theimplementationofpotentialsensitivity

a- Micro tubes strip before inoculated bacterial growth

b-Microtubesstripafterinoculatedbacterialgrowth

Fig.1Antibioticsusceptibilitytestperformingmicrotubesstripbefore(a)andafter(b)inoculatedbacterialgrowth.
Bluecolor:sensitive;Yellow:resistancebacteria.

testingandprovidingofbestservices,aswellasreducingthecostandmaterials,alltheserequiredforeconomysupporttoachievesensitivitytestforbacterialtypes.Recognizabletotaperecordalsoreducedtheperiodrequiredtoachievethedesiredgoalof24hoursto2hoursandthisreducestheeffortandincreasesthespeedofthedeliveryoftherequiredtreatmenttothepatient.Finally,werecommendtheMinistryofHealthfortheadoptionofthewaytoensurethetestrequiredinalldiagnosticlaboratories.

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تطوير طريقة جديدة لفحص حساسية البكتريا المرضية لبعض للمضادات الحياتية

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الخلاصة

طورت طريقة جديدة لفحص حساسية البكتريا للمضادات الحياتية في المختبرات الطبية وذلك باستخدام شريط الفحص (المكون منأنابيب أو جيوبصغيرة لاصقة بالشريط) يحتوي في داخلهعلى الوسط الزرعيالمجفدالمتكون من مرقصويا التربتون، **5%** كلوكوزو**0.1%**صبغة البروموثايمول الزرقاء وأحد المضادات حياتية **(Ampicillin, Tetracycline, Chloramphenicol)** وبتراكيز نموذجية (التركيز الأدنى المثبطة للنمو **MIC,**). استخدمت عالق العزلات البكتيرية القياسية (***Staphylococcus aureus, Escherichia coli*** و***Pseudomonas*aeruginosa**) في الاختبار وتلقيح الوسط ، بعد أن ضبطت عكرة العالق مع عكرة محلول ثابت العكرة القياسيمكفرلاند **0.5** **(1** × **10^6** وحدة تكوين المستعمرات / لمل الواحد (**CFU/ ml**) وحضنت لفترة ساعتين في**^oC 37**.

حددت التركيز الأدنى (**MIC**)المثبط للمضادالحياتي **Ampicillin** لأنواع البكتريا ***Staph. aureus, E. coli*) *Pseudomonas aeruginosa,*** **4، 32، 256**مايكروغرام لكل مليليتر ولأنواع البكتريا على توالي.وكانت التركيز الأدنى للمضاد الحياتي **Tetracycline** لأنواع البكتريا **512،512و32**مايكروغرام لكل مليليتر على التوالي. في حين كان التركيز الأدنى للمضاد **Chloramphenicol512** ، **32** و **512** مايكرو غرام لكل مليليتر على التوالي.

كما لوحظت إن العزلات البكتيرية المقاومة للمضادات الحياتية تمكنت من النمو وتخمير سكر الكلوكوز والذي سببت في تغير لون الوسط من اللون الأزرق إلى الأصفر نتيجةتغير الـ**pH**، بينما البكتريا الحساسة لم تنمو ، لذا لم تحدثتغير في اللون.قورنت نتائج البحث مع طريقة أقراص المضادات الشائعة استخداما وكانت النتيجة مطابقة.
تميزت هذه الطريقة الجديدة كونها سريعة (ساعتين فقط) وذات كلفه قليله جدا مقارنة بالتقنيات التقليدية. كما يميز شريط الفحص المبتكر بالاستقرار العالية (أكثر من سنه واحده) وحساسيتها في التشخيص العديد من البكتريا المرضيةضمنا العزلات البكتيرية القياسية مقارنة بالطرائق التقليدية.

الكلمات المفتاحية : الحساسية، MIC**، المضادات، تقنيات المختبرات الطبية**