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Diagnostic Utility Of Various Phenotypic Tests For Metallo-β-Lactamase Detection In Carbapenem Resistant *Pseudomonas aeruginosa* At A Tertiary Care Hospital

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Abstract:

Introduction: Carbapenems groups of antibiotics are used for the treatment of *Pseudomonas aeruginosa* infections. Metallo-β-lactamases are able to efficiently hydrolyze these classes of drugs. Early detection of the MBL-producing *P. aeruginosa* is necessary to prevent spread of resistance in community and also to treat patient accurately.

Aim: This study was undertaken to determine the antibiotic resistance pattern of carbapenem resistant *P. aeruginosa* and to identify Metallo-β-lactamase in clinical isolates of Carbapenem resistant *P. aeruginosa* (CRPA) by phenotypic methods.

Materials & **Methods**: An observational cross sectional study was done in the Department of Microbiology, S.M.S. Medical College, Jaipur for two years. Various clinical samples received from patients were cultured and *P. aeruginosa* were identified as per standard protocol. Antimicrobial susceptibility testing was done according to CLSI guidelines. Total 199 Carbapenem Resistant *P. aeruginosa* isolated from various clinical samples were further evaluated for MBL production by Combined Disc Diffusion Test, Double Disc Synergy Test and Epsilometer test.

Results: Total 945 *P. aeruginosa* were isolated, out of 945, 199 Carbapenem Resistant *P. aeruginosa* were recovered. Out of 199 CRPA, 136 (68.3%) were found MBL producers by CDDT, 122 (61.3%) by DDST while 139 (69.8%) were found positive by E test.

Conclusion: Our study concludes that MBL production is an important mechanism in Carbapenem Resistant *P. aeruginosa* (CRPA). Combined disc diffusion test will be helpful toward large-scale monitoring of these emerging resistant isolates. All the isolates should be routinely screened for MBL production.

Key Words: Carbapenems, Metallo β- Lactamase, *Pseudomonas aeruginosa*

Introduction: *Pseudomonas aeruginosa* is one of the most significant microorganism responsible for nosocomial infections, ranges from urinary tract infections to severe sepsis[1]. Due to inappropriate use of antibiotics, *Pseudomonas aeruginosa* has become resistant to multiple antimicrobial agents[2]. Carbapenems have a broad spectrum of antibacterial activity and are used as last option drugs for the treatment of infections caused by multi drug resistant *P. aeruginosa*[3]. So, Carbapenem resistance among *Pseudomonas aeruginosa* has been a major concern[2]. Carbapenem resistance in *P. aeruginosa* occurs due

to diminished outer membrane permeability, increased efflux system, modification of penicillin-binding proteins and Carbapenem hydrolysing enzymes Carbapenemases[4].

A variety of transferable β-lactamases have been found in clinical isolates of *P. aeruginosa* around the world. Among them, Carbapenemases are of major clinical importance because they inactivate carbapenems together with other β-lactams.

Carbapenemases includes Class A clavulanic acid inhibiting enzyme, Class B Metallo-β-lactamase (MBL) and Class D oxacillinase[5]. Class B type of carbapenemase, require bivalent metal ions, usually zinc for their activity. It is the most common resistance mechanism to carbapenem in *P. aeruginosa*[6, 7].

Pseudomonas aeruginosa, producing MBLs, was first reported from Japan in 1991 and since then has been reported from various parts of the world[(8,9)]. *Pseudomonas aeruginosa* owning MBLs constitute nearly 20% of all nosocomial isolatesin some countries[10]. Prevalence of MBLs ranges from 7-65 % in India[6,7,11]. Many phenotypic methods have been described the detection of MBL producing bacteria. These methods are based on the ability of metal chelators, such as EDTA and thiol-based compounds, to inhibit the activity of MBLs[12]. Furthermore, MBLs are fixed by genes that acquired by transfer of mobile genetic elements. Acquired MBL gene can be spread among various strains of bacteria such as *P. aeruginosa*. Approximately nine different types of acquired MBL genes have been identified. The most important types that contribute to epidemiological and clinical importance are the IMP, VIM, SPM and NDM type enzymes[13]. This study was undertaken to determine the antibiotic resistance pattern of carbapenem resistance *P. aeruginosa* and to identify Metallo-β-lactamase in clinical isolates of Carbapenem resistant *P. aeruginosa* (CRPA) by phenotypic methods.

Material & Methods: A cross sectional observational study was conducted in the Department of Microbiology, Sawai Man Singh Medical College & Hospital, Jaipur from June 2016 to May 2018. Ethical approval was taken from the Institutional Ethical Committee before the commencement of the study. Various clinical samples, received from patients admitted in the wards, outpatient department (OPDs) and Intensive Care Units (ICUs) were cultured and *P. aeruginosa* were identified as per standard protocol[14]. Antimicrobial susceptibility testing was done on Muller Hinton Agar by Kirby Bauer disc diffusion method according to CLSI guidelines (M100-S26) 2016[15]. ATCC 27853 *Pseudomonas aeruginosa* was used as quality control strain. Carbapenem Resistant *P. aeruginosa* were defined as isolates found resistant to Meropenem (10µg) or / and Imipenem (10µg) and they were further evaluated for MBL production by following tests.

Combined Disc Diffusion Test (CDDT): This test was performed as described by Yong et al. A lawn culture of test strains (0.5 McFarland's opacity standards) was done on Muller Hinton Agar. Two antibiotic discs, one is Imipenem (10 µg) alone and another disc Imipenem + EDTA in combination were placed. After overnight incubation, if the zone of inhibition of Imipenem + EDTA discs compared to Imipenem alone is >7 mm, the test was considered as positive for Metallo-β- Lactamase[16].

Double Disc Synergy Test (DDST): this test was performed as described by Lee et al. Test strains were inoculated (0.5M McFarland's Standard) on Mueller Hinton agar. An Imipenem (10µg) disc were placed 20 mm center to center from another EDTA disc (750 µg). Enhancement of the inhibition zone in the area between Imipenem and the EDTA disc in comparison with the inhibition zone on the far side of the antibiotic disc was considered positive MBL producer[17].

MBL E Test: A lawn culture of test strains (0.5 McFarland opacity standards) was done on Muller Hinton Agar. E test strips with IMP (4 to 256 µg/ml) and IMP EDTA (1 to 64 µg/ml) was purchased from biomeriux distributer and they were inoculated on Muller Hinton agar and after overnight incubation at 37°C. MIC was taken as the point of intersection where ellipse was formed on E test strip. The strain was interpreted positive for MBL if ratio of imipenem MIC / imipenem EDTA MIC ≥ 8. Phantom zone formation or imipenem ellipse deformation was also consideredas MBL positive.

Statistical Analysis: Data were entered in MS excel (2010) and appropriate statistical calculations were done. Test characteristics sensitivity, specificity,

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positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated.

Results: Total 945 *P. aeruginosa* were recovered. Out of which 199 Carbapenem Resistant *P. aeruginosa* (CRPA) were isolated, out of which 56 (28.1%) were isolated from Pus, 47 (23.6%) from Urine, 31 (15.6%) from Burn swab, 31 (15.6%) from Tracheal swab, 12 (6.0%) from Sputum, 5 (2.5%) from Blood and 17 (8.5%) were from other clinical samples.

All CRPA isolates were sensitive to Polymyxin and Colistin. Highest resistance was observed to Piperacillin 187 (94.0%) followed by Aztreonam 176 (88.4%), Ceftazidime 163 (81.9%), Ciprofloxacin 143 (71.9%), Gentamycin 134 (67.3%) and Tobramycin 131 (65.8%). 92 (46.2%) isolates were Imipenem resistant (IRMS), 32 (16.1%) were Meropenem resistant (ISMR) and 75 (37.7%) strains were resistant to both carbapenems (IRMR). [Table/Figure 1].

Among 199 CRPA, 136 (68.3%) strains were MBL positive by CDDT, 122 (61.3%) by DDST while 139 (69.8%) were confirmed positive by E test [Table/Figure2].

Out of 199 isolates, 105 CRPA were found to be MBL producer by all 3 methods. 139 isolates were detected as confirmed MBL producers by standard epsilometer test (E Test). Total 136 isolates were found MBL producer by CDDT among them 134 were found true positive and 2 were false positive in concordance with standard E test while by DDST 122 isolates were found MBL producer among them 105 isolates were true positive and 17 were false positive [Table/Figure3]. Sensitivity, specificity and accuracy for CDDT was found 96.4%, 96.7% and 96.5% respectively while for DDST sensitivity 88.2%, specificity 71.7% and accuracy 82.7% was observed [Table/Figure4].

When we evaluated diagnostic agreements between the phenotypic tests, 'almost perfect agreement' was observed between CDDT and E test with kappa coefficient 0.918 and CDDT and DDST ($\kappa = 0.864$) while there was 'substantial agreement' between DDST and E test ($\kappa = 0.607$).

Discussion: *Pseudomonas aeruginosa* is one of the most important causative agents of nosocomial infections. Antibiotic resistance among *P. aeruginosa* is one of the major problems in treating hospitalized patients. Carbapenems are the last drug of choice for the treatment of drug resistant *P. aeruginosa* infections due to the stability of these agents against the majority of β-lactamase. MBL production is major beta lactamase resistance mechanism, responsible for carbapenem resistance, but increasing resistance to these antibiotics, has limited their effectiveness.

It has been observed that *P. aeruginosa* mainly causes surgical site infections; open wound infections followed by urinary tract infections.[18]A total 199 CRPA were recovered in our study. Maximum CRPA was mainly isolated from pus (23.6%)followed by urine, burn swab, tracheal swab, sputum and blood. In a study of Lavanya et al also reported that *P. aeruginosa* was mainly isolated from pus samples (82%)followed by urine, blood and sputum[19]. In a study of Arunagiri K et al, maximum CRPA was isolated from Urine samples[2]. This difference could be due to different study environments under which study were performed. In our study CRPA showed 46.2% and 16.1% resistance to Imipenem and Meropenem respectively and resistance to both carbapenems was observed in 37.7% isolates. In several studies across the world, variable rates of resistance (4-60%) have been reported for Imipenem and Meropenem[20]. Gladstone et al reported 42.8% carbapenem resistance among *P. aeruginosa* isolates[21]. Carbapenem resistance may vary it depends on the clinical use of these antibiotics in different clinical settings. Many studies have reported high resistance to Meropenem in compare to Imipenem[22, 23, 24]. In our study Imipenem resistance was observed more in comparison to Meropenem resistance, although Imipenem is less used for treatment of *P. aeruginosa* infections. But interestingly, Imipenem resistant isolates were mostly MBL-producers in our study.

In our study, maximum resistance to CRPA was observed to Piperacillin 187 (94.0%), followed by Aztreonam 176 (88.4%), Ceftazidime 163 (81.9%), Ciprofloxacin 143 (71.9%), Gentamycin 134 (67.3%) and Tobramycin 131 (65.8%). Pitout et al reported 78% resistance to Ceftazidime, 86% to Gentamycin, 73% to Piperacillin and 55% to Ciprofloxacin[12]. This study showed high antibiotic resistance that was similar to our study. CRPA showed high resistance to other antimicrobials. This could be due to co-

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existence of genes encoding drug resistance to those antibiotics on the plasmids carrying MBL genes. Fused gene cassettes carrying MBL gene and an aacA4 gene that encodes aminoglycoside resistance are also known to exist[25]. However resistance to different antibiotics was dependent on the origin of the strains, possibly reflecting the patterns of antibiotic usage in the hospital.

In our study the most effective drug was Polymyxin B and Colistin effective on 100% of isolates. Similar findings were observed by Franco et al (100% sensitivity to Polymyxin)[26].

MBLs production in *Pseudomonas aeruginosa* was first reported from Japan in 1991. Since then, it has been described from Asia, Europe, Australia, South America and North America[27]. Since then the incidence of MBL is increasing among CRPA. Navneeth et al first reported 12% MBL production by CRPA in India[28].A study conducted by Mary et al, reported 42% MBL production by Pseudomonas aeruinosa[29] whereas in our study, 139 (69.8%) CRPA were found positive for MBL production which is similar with the study of K arunagiri et al reported 70.1% MBL production in Carbapenem Resistant *P. aeruginosa*[2]. In a study of A. Manoharan et al reported 42.6% MBL production[6] which is lower than from our study.

We have found that out of 199 CRPA, only 139 (69.8%) strains were MBL producers. In a study of Nandy, S. et al also reported 48.8% carbapenem resistance among which only 40.5% isolates were MBL producers[30]. This indicates that other resistance mechanisms such as loss of oprD porin, change in outer membrane permeability and by active efflux pump[31] is more frequent resistance mechanisms towards carbapenem resistance.

For MBL detection, E test was used as a gold standard test which is a quantitative and sensitive test but due to cost constraints it is not possible to perform practically. Other phenotypic tests CDDT and DDST were also performed and compared with E test. The efficacy of CDDT was found to be equally comparable to that of MBL E test. The sensitivity of double disc synergy test was found to be quite lower (88.2%) than the other 2 tests which is comparable with the study of Nandy, S. et al.[30] and Vaishali, G. et al[32] also reported lower sensitivity of DDST (82%) than CDDT (100%).

In a study of A. Lucena et al[33] reported that MBL producing CRPA showed high resistance to other group of antibiotics in compare to non MBL producing isolates. We also reported that MBL positive isolates show a very high resistance to various groups of drugs other than β-lactams. This indicates that most of the MBL producing isolates included in our study were carrying multi drug resistance genes.

The incidence of MBLs has been increasing slowly and it is circulating worldwide by mobile genetic elements. The detection of MBL is most significant in deciding the most suitable therapeutic agents.

Conclusion: MBL production in carbapenem resistant *Pseudomonas aeruginosa* was found to be 69.8% (139/199) of total isolates. The increasing frequency of an MBL-positive isolates in a hospital setting poses a therapeutic problem, as well as a serious concern for infection control management. So, with routine antibiotic sensitivity, early detection of these β- lactamase has necessitates preventing further spread of resistance. For the screening of metallo beta lactamase, Combined Disc Diffusion Test was found more sensitive and specific than Double Disc Synergy Test and have comparable agreement with standard E Test. Screening of MBLs by CDDT will guide in therapeutic alternative of antibiotic and to institute the appropriate antimicrobial agent to the patient and to prevent the spread of MBL positive organisms.

Limitations: Only MBL carbapenemases were detected, non MBL carbapenemase were not detected in our study. Confirmatory test, PCR was also not included in this study. We studied only carbapenem resistant isolates as it is most often preferred; however screening of all isolates should be done as MBL is also reported in carbapenem sensitive isolates.

Recommendations: We recommend that all isolates of *P. aeruginosa* resistant to Imipenem, Meropenem and ceftazidime should be routinely screened for as MBLs are reported in these isolates. CDDT test is simple to perform and interpret. It is performed as routine antimicrobial susceptibility method as it can be easily introduced into the routine work of a clinical laboratory. It is less expensive than the MBL E-test.

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References-

1. Todar K. Todar's Online Textbook of Bacteriology [internet]. Madison: University of Wisconsin, Department of Bacteriology; 2004. Pseudomonas. Available from: http://textbookofbacteriology.net/pseudomonas.html.
2. Arunagiri K, Sekar B, Sangeetha G, John J. Detection and Characterization of Metallo-β-lactamases in *Pseudomonas aeruginosa* by Phenotypic and Molecular Methods from Clinical Samples in a Tertiary Care Hospital**.** West Indian Med J. 2012; 61(8):779-783.
3. Meletis G, Exindari M, Vavatsi N, Sofianou D, Diza E. Mechanisms responsible for the emergence of carbapenem resistance in *Pseudomonas aeruginosa*. Hippokratia. 2012; 16 (4):303-307.
4. Livermore DM. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare? Clin Infect Dis. 2002; 34(5):634–40.
5. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother. 1995; 39(6):1211–33.
6. Manoharan A, Chatterjee S, Mathai D; SARI Study Group. Detection and characterization of metallo beta lactamases producing *Pseudomonas aeruginosa*. Indian J Med Microbiol 2010; 28:241-4.
7. Bush K. ß-Lactamases of increasing clinical importance. Curr Pharm Des1999; 5: 839-45.
8. Gales AC, Menezes LC, Silbert S, Sader HS. Dissemination in distinct Brazilian regions of an epidemic carbapenem-resistant *Pseudomonas aeruginosa* producing SPM Metallo-ß-lactamase. J AntimicrobChemother2003; 52: 699-702.5.
9. Lee K, Lee WG, Uh Y, Ha GY, Cho J, Chong Y. VIM- and IMP-type metallo-ß-lactamase-producing Pseudomonas spp. and Acinetobacter spp. in Korean hospitals. Emerg Infect Dis 2003; 9: 868-71.6.
10. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo β-lactamases: the quiet before the storm? ClinMicrobiol Rev2005; 18: 306-25.
11. Behera B, Mathur P, Das A, Kapil A, Sharma V. An evaluation of four different phenotypic techniques for detection of metallo-β-lactamase producing *Pseudomonas aeruginosa*. Indian J Med Microbiol 2008; 26: 233-7.
12. Pitout JDD, Gregson DB, Poirel L, McClure JA, Le P, Church DL. Detection of *Pseudomonas aeruginosa* producing metallo-β-lactamases in a large centralized laboratory. J ClinMicrobiol 2005; 43:3129-35.
13. Dong F, Xu X-W, Song W-Q, Lü P, Yu S-J, Yang Y-H et al. Characterization of multidrug-resistant and metallo beta lactamase producing *Pseudomonas aeruginosa* isolates from a paediatric clinic in China. Chin Med J2008; 121:1611−6.
14. Govan, J.R.V. (1996). Pseudomonas, Stenotrophomonas, Burkholderia. In: Collee, J.G., Fraser, A.C., Marmion, B.P., Simmons, A.(eds). Mackie & McCartney Practical Medical Microbiology. Churchill Livingstone, New York, USA, p.413-424.
15. CLSI. CLSI Supplement M100S. 26th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2016. Performance Standards for Antimicrobial Susceptibility Testing.
16. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo-beta-lactamase-producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. J ClinMicrobiol2002;40:3798-801.
17. Lee K, Lim Y. S, Yong D, Yum J. H, & Chong Y. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-β-lactamase-producing isolates of Pseudomonas spp. and Acinetobacter spp. Journal of Clinical Microbiology. 2003; 41(10): 4623-4629.
18. Toniolo A, Endimiani A, Luzzaro F. Microbiology of postoperative infections. Surg Infect.2006; 7 suppl 2: S 13-16.
19. Lavanya M, Thangam M. Coexistence of metallo-beta-lactamase-encoding genes in

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Pseudomonas aeruginosa. Indian J Med Res. 2017; 46-52.

20. Gonlugur U, Bakiri MZ, Akkurt I, Efeoglu T. Antibiotic susceptibility patterns among respiratory isolates of gram negative bacilli in a Turkish University Hospital. BMC Microbiol 2004; 4:32-6.
21. Gladstone P, Rajendran P, Brahmadathan KN. Incidence of carbapenem resistant non fermenting gram-negative bacilli from patients with respiratory infections in the intensive care unit. Indian J Med Microbiol 2005; 23:189-91.
22. Taneja N, Aharwal SM, Sharma M. Imipenem Resistance in non fermenters causing nosocomial urinary tract infection. Indian J Med Sci 2003; 57: 294-9.
23. Ramakrishnan K, Rajagopalan S, Nair S, Kenchappa P, Chandrakesan SD. Molecular characterization of metallo β-lactamase producing multidrug resistant *Pseudomonas aeruginosa* from various clinical samples . Indian J Patho Microbiol 2014; 57:579-82.
24. Nordmann P, Poirel L. Emerging carbapenemases in gram-negative aerobes. Clin Microbial. Infect 2002; 8:321-31.
25. Bajpai V, Govindaswamy A, Khurana S, Batra P, Aravinda A, Katoch O, Hasan F, Malhotra R & Mathur P. Phenotypic & genotypic profile of antimicrobial resistance in Pseudomonas species in hospitalized patients. Indian J Med Res February 2019; 216-221.
26. Franco B E, Altagracia Martínez M, Sánchez Rodríguez MA, Wertheimer AI. The determinants of the antibiotic resistance process. Infect Drug Resist 2009; 2:1-11.
27. Yano H, Kuga A, Okamota R, Kitasato H, Kobayashi T, Inon M. Plasmid coded Metallobetalactamase (imp 6) conferring resistance to carbapenems, especially meropenam. Antimicrob Agents Chemother 2001; 45:1343-8.
28. Navneeth BV, Sridaran D, Sahay D, Belwadi M. A preliminary study of metallo-β-lactamase producing *Pseudomonas aeruginosa* in hospitalized patients. Indian J Med Res 2002; 116: 264-8.
29. Jesudason MV, Kandathil AJ, Balaji V. Comparison of two methods to detect carbapenemase and metallo-β-lactamase production in clinical isolates. Indian J Med Res 2005; 121:780-3.
30. Nandy S, Das AK, Dudeja M. Prevalence of metallo beta lactamase in clinical isolates of *Pseudomonas aeruginosa* in a tertiary care hospital. Int J Community Med Public Health 2015; 2: 566-9.
31. Deshpande P, Rodrigues C, Shetty A, Kapadia F, Hedge A, SomanR. New Delhi metallo-betalactamase (NDM-1) in Enterobacteriaceae: treatment options with carbapenems compromised. J Assoc Physicians India 2010; 58:147–9.
32. Vaishali G, Renu B, Vaishali D. Different Methods of Detection of Metallo beta lactamase Producing *Pseudomonas aeruginosa* from Tertiary Care Centre. Int J Sci Stud 2015; 2(12):155-159.
33. Andréa L, Libera M. Dalla C et al. Comparison of phenotypic tests for the detection of metallo-beta-lactamases in clinical isolates of *Pseudomonas aeruginosa* Enferm Infecc Microbiol Clin. 2014; 32(10):625–630. Lucena A, Costa L.M.D, et al. Enferm Infecc Microbiol Clin. 2014; 32(10):625–630

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Table 1: Antibiotic resistance pattern of Carbapenem Resistant *P. aeruginosa*

Antibiotics	CRPA = 199
Piperacillin (100µg)	187 (94.0%)
Piperacillin/Tazobactam (100/10µg)	65 (32.7%)
Gentamicin (10µg)	134 (67.3%)
Tobramycin (10µg)	131 (65.8%)
Ciprofloxacin (5µg)	143 (71.9%)
Norfloxacin (10 µg) only for urine	20/47 (42.6%)
Aztreonam (30µg)	176 (88.4%)
Ceftazidime (30µg)	163 (81.9%)
Imipenem (10µg)*	167 (83.9%)
Meropenem (10µg)*	107 (53.8%)

* 75 strains were resistant to both the carbapenam (Imipenem and Meropenem)

Table 2: MBL detection among Carbapenem Resistant *Pseudomonas aeruginosa* by phenotypic methods (N= 199)

Methods	No. of Positives & pecencentage

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CDDT	136 (68.3%)
DDST	122 (61.3%)
E Strip Test	139 (69.8%)

Table 3: Comparison of phenotypic tests (CDDT, DDST) with Standard E strip for MBL production (N= 199)

Test Name	MBL +ve with E test (139)		MBL -ve with E test (60)	
	POSITIVE (TP)	NEGATIVE (FN)	POSITIVE (FP)	NEGATIVE (TN)
CDDT	134	5	2	58
DDST	105	14	17	43

Table 4: Test characteristics of phenotypic tests (CDDT and DDST) with Standard E Test for MBL production.

Test Name	Sensitivity	Specificity	PPV	NPV	Accuracy
CDDT	96.4%	96.7%	98.5%	92.1%	96.5%
DDST	88.2%	71.7%	86.1%	75.4%	82.7%

Table 5: Diagnostic agreement between phenotypic tests CDDT, DDST and E Test for MBL detection

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Test Name	Kappa Coefficient	SE of Kappa	95% Confidence Interval	Agreement	Interpretation
CDDT vs. E Test	0.918	0.031	0.858 to 0.978	96.5 %	Almost perfect agreement
DDST vs. E Test	0.607	0.063	0.482 to 0.731	82.7 %	Substantial agreement
CDDT vs. DDST	0.864	0.038	0.790 to 0.938	93.97 %	Almost perfect agreement

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