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Microbiology

Identification and Detection of Enterococci using Conventional methods and its comparison with commercial system MicroScan (Autoscan4).

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ABSTRACT Background:-The frequency of antimicrobial resistant Enterococci is increasing, making accurate identification and antimicrobial susceptibility important. Antimicrobial susceptibility testing using conventional and automated technique both are commonly used throughout the country. Comparison studies of both are available for Enterobacteriaceae etc but for Enterococci such study are few. Hence a comparison in our settings was needed. Aim:- To find out the commonest species of Enterococci and comparison of its antibiogram profile obtained by commericial system Microscan (Autoscan -4) using positive combo panel 20.Material and Methods:- A total of, 120 Enterococcal isolates obtained over a one year period were studied. They were evaluated, using conventional and minimum inhibitory concentration (MIC) method. These isolates were mainly from urine 91 (76%) and remaining from blood 29 (24%). Isolates were identified using conventional identification method first and then the same isolates were subjected to antimicrobial susceptibility test by both the methods simultaneously. A comparison of identification and the antibiogram obtained by both the methods was done. Result:- The most common findings in our clinical settings by both the methods was E.faecalis 66(55%), E.faecium 32 (26.67%), E.raffinosus 9 (7.5%), E. avium 7 (5.83%) & E.pallens 6 (5%). The most common susceptible antibiotics were Ampicillin (72%),Penicillin G (58%), Linezolid (39%) , Tetracycline (21%) Norfloxacin(10%). Conclusion: - In Our study we found that the susceptibility pattern is same for both Microscan and Kirby Beaur's disc diffusion method but in MicroScan few database for antimicrobial agents is not available.

KEYWORDS : MicroScan, Conventional method, Enterococci.

Introduction:

Enterococcus, an indigenous flora of the intestinal tract, is known to be relatively a virulent in healthy individuals, but they behave as pathogens in hospitalized patients [1, 2]. They have emerged as nosocomial pathogens in spite of low levels of their virulence [2,3].

Automated systems, including MicroScan (AutoSCAN-4) system, have been developed to identify and to determine the antimicrobial susceptibility of enterococci. The common species of *Enterococcus* which cause human infections are *E.faecalis* (80-90 %) and E.*faecium* (5-10%)[4], but now there is an increase in the isolation rate of E faecium and other species from various clinical samples [1,2]. Previous studies have shown conventional MicroScan panels to be reliable in the identification of *Enterococcus* species, even though the data bank includes only *E. faecalis, E.faecium*, *E. raffinosus, E. avium* and *E.pallens* There are also conflicting reports on the reliability of the MicroScan system to detect strains of *Enterococcus* species [5, 6].

Methods & Materials:

The present study was conducted in the Department of Microbiology G. R Medical College Gwalior and Acer Labs Gwalior, Madhya Pradesh, India. The isolates of Enterococcus from throat swabs, sputum, vaginal swabs and stool were excluded from the study, as they formed a part of the normal flora[4].

One hundred twenty enterococcal isolates were evaluated, including conventional and minimum inhibitory concentration (MIC) method. Isolates of *Enterococci* identified in the Clinical Microbiology Laboratory were 91 from urine and 29 from blood samples. Identified isolates of *Enterococcus* is 66 *E.faecalis*, 32 *E.faecium*, 9 E.raffinosus, 7 *E.avium,* 6 *E.pallens*. Prior to testing, the isolates were passed twice on sheep blood agar to ensure a pure culture. They were identified by using standard tests like colony morphology, gram staining, catalase test, bile esculin test, salt tolerance test and α- pyrrolidonyl β- naphthylamide test (PYR test) [4,7,8]. Their speciation was on the basis of the sugar fermentation test (Facklam and Collin) [9], growth in pyruvate broth, arginine hydrolyzing property and motility and pigment production [4, 9, 10]. The antimicrobial susceptibility testing was performed by the Kirby Bauer disc diffusion method on Muller Hinton agar as per CLSI guidelines[11]. Ethtical clearance not required as study was on routine laboratory isolates.

MicroScan panels:

Conventional MicroScan panels (Positive BreakpointCombo Type 20) were inoculated with fresh isolates by the turbidity standard technique. The panels were incubated for a full 24 hrs at 370C & read with the MicroScan (AutoSCAN-4) reader. All procedures were performed according to the manufacturer's directions.

Conventional Biochemicals:

Following tested were performed on all enterococcal isolates: tolerance to and hydrolysis of bile-esculin; growth in brain heart infusion broth with 6.5% NaCl; deamination of arginine (1%) in Moeller decarboxylase base; fermentation of 1% lactose, 1% mannitol, 1% sorbose, 1% glucose, 1% sucrose, 1% raffinose, and 1% arabinose in heart infusion Broth ; and motility at 370C . We performed PYR (pyrrolidonyl-arylamidase) test, the ability of this test is hydrolysis of L-naphthylamide-β- naphthylamide by producing aminopeptidase enzyme & after addition of PYR reagent,

which is positive shows deep cherry red & negative shows yellow-orange colour.

These tests differentiate in between *Enterococcus faecalis* & *Streptococcus agalactae.* Identification scheme included the following differentiations: *E. faecalis* gives Arabinose (-) & glucose (+), *E.faecium* gives Arabinose & glucose (+), *E.raffinosus* gives both sugars are positive & ADH (Arginine dihydrolase) (-), *E.pallens* keep PYR & ADH are negative, *E.avium* gives raffinose, ADH (-) & glucose (+).

Antimicrobial Susceptibility testing:

Susceptibility testing of five antimicrobial agents (Ampicillin, Linezolid, Norfloxacin, PenicillinG and Tetracycline) was performed by the disc diffusion assay on Mueller Hilton Agar plates. These antimicrobial agents were choosen as per routine clinical trends and for easier comparison with Microscan (AutoSCAN) result. After 18-24 hrs of incubation at 370 c, inhibition zone diameters around each disc were measured & the diameter of inhibition zones was interpreted according to the criteria recommended by the CLSI[11].

Result:

Enterococci which were isolated from all 4000 clinical samples were 120 accounting for an infection rate of 3%. The maximum number of *Enterococcus* isolates were obtained from urine-91 (76%), followed by blood-29 (24%). In urine isolates *E. faecalis* amounted to 56(46.67%) infections, *E.faecium* to 14(11.67%) infections, *E. raffinosus* to 14(11.67%) & *E.avium* to 7(5.83%) but in blood isolates ratio of *E. faecalis* amounted to 11(9.17%) & *E.faecium* to 18(15%) infections. All 120 isolates were correctly identified by *MicroScan* panels and on the basis of conventional biochemical characterization shows (Table no.-1).

TABLE No.1. – Identification of Enterococcus species is accomplished by Biochemical & Physiologic tests:-

Species	Growth BE Agar	Growth 6.5% Nacl	LAP	PYR	MOT	ADH	ACID PRODUCED FROM						
							GLC	MNTL	SORB	ARAB	SBTL	RAF	SUC
E.avium	+	+	+	+	-	-	+	+	+	+	+	-	+
E.pallens	+	+	+	-	-	-	+	+	+	+	+	+	+
E.raffinosus	+	+	+	+	-	-	+	+	+	+	+	+	+
E.faecalis	+	+	+	+	-	+	+	+	-	-	+	-	+
E.faecium	+	+	+	+	-	+	+	+	-	+	V	V	+

Bile-esculinagar(BEAgar), Leucineaminopeptidase(LAP), Pyrrolidonylarylamidase(PYR), Motility(MOT), ArginineDihydrolase(ADH), Glucose(GLU), Mannitol(MNTL), Sorbose(SORB), Arabinose(ARAB), Sorbitol(SBTL), Raffin ose (RAFF), Sucrose(SUC).

In our study we find out that MIC Antibiotic Susceptibility pattern is same as Kirby Beaurs Disc diffusion method. These isolates were sensitive to Ampicillin 87(72%), Linezolid 47(39%) Norfloxacin 12(10%), PenicillinG 70(58%) & Tetracycline 26(21%) show (Table no.-2).

TABLE No. 2:- Antibiotic susceptibility pattern of Enterococci:-

Name of Organism	Number of Organism	Ampicillin		Linezolid		Norfloxacin		PenicillinG		Tetracycline	
		S	R	S	R	S	R	S	R	S	R
E. faecalis	66	50	16	16	50	8	58	50	16	0	66
E.faecium	32	24	8	16	16	0	32	8	24	8	24
E.raffinosus	9	5	4	7	2	0	9	3	6	6	3
E.avium	7	4	3	3	4	2	5	6	1	6	1
E.pallens	6	4	2	5	1	2	4	3	3	6	0

Abbreviations: S- susceptible; R- resistant;

Note:- Incase of Amox/Clav, Chloramphenicol, Ciprofloxacin, Erythromycin, Gentamycin & Vancomycin etc antibiotics are resistant to all Enterococcus species.

Discussion:-

The *Enterococcus* species have now emerged as nosocomial pathogens. Hence, it is important to know the changing patterns of the *Enterococcus* infections and the antimicrobial susceptibility patterns of the isolates [1]. The ability to accurately identify enterococci at the species level is important not only for epidemiological purposes but also to recognize species such as *E. faecium* which tend to show resistance to antimicrobial agents commonly used for therapy. These results are consistent with those of a recent study confirming the ability of the conventional MicroScan panels, which were used exclusively by laboratories in our Study, to detect selective antibiotics susceptible in enterococci.

No beta-lactamase-positive enterococci were identified in this study, the reliability of conventional MicroScan panels for detecting strains which produce this enzyme is not known.

The results of this study confirm that the conventional MicroScan Positive Breakpoint Combo Type 20 panels are reliable in identifying species of Enterococci to one extent but supplemental biochemical testing in combination with MicroScan panel was needed to correctly identify these species.

Regarding the Susceptibility of commonly used antimicrobial agents, our study showed that 72% were susceptible to Ampicillin, 58% to Penicillin, 39% to Linezolid, 21% to Tetracycline & 10% to Norfloxacin.

One interested finding was the lack of result in (Autoscan 4) positive combo panel 20 for many antimicrobial agents like Cephalothin, Clindamycin, Fosfomycin, Oxacillin etc for 100% of enterococci isolates in MIC testing probably because the system do not have data base for these antimicrobial agents.

Conclusion:

By our study, we concluded that the conventional system MicroScan (Autoscan-4) is reliable for find out the species of enterococci to enlarge trend but even those some additional biochemical test are required for correct species identification. The antibiogram pattern obtained with Kirby Beaur's disc diffusion method also coincides with the MIC pattern for five antimicrobial agents. Despite the cost of conventional system (Autoscan-4) it can be used for routine antimicrobial susceptibility for small and medium laboratories.

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Reference:

1. Desai PJ, Pandit D, Mathur M, Gogate A. The prevalence, identification and the distribution of various species of enterococci which were isolated from clinical

samples, with special reference to the urinary tract infections in catheterized patients. India J Med Microbial. 2001; 19:132–37.
2. Murray BE. The life and the times of the Enterococci. Clin Microbiol Rev. 1990; 3:46–65.
3. Vandamme P, Vercauteran E, Lammens C, Pensart N, Ievan M, Pot B, et al. A survey on the enterococcal susceptibility pattern in Belgium. J Clin Microbial. 1996; 34:2572–76.
4. Ross PW. 14th edition. Elsievier: 2006. Streptococci and Enterococci. Mackie and Mccartney's Practical Medical Microbiology, pp. 268–69.
5. Louie, M., A. E. Simor, S. Szeto, M. Patel, B. Kreiswirth, and D. E. Low et al Susceptibility testing of clinical isolates of Enterococcus faecium and Enterococcus faecalis. J. Clin. Microbiol. 1992;30:41–45.
6. Tritz, D. M., P. C. Iwen, and G. L. Woods, et al Evaluation of MicroScan for Identification of Enterococcus species. J. Clin. Microbiol. 1990;28:1477–1478.
7. Koneman EW, Allen S, Janda WM, Schreckenberger PC, Winn WC. 6th edition. Baltimore Lippincott William and Wilkins; 2006. Isolation and Identification of Streptococci and Streptococci like organisims. Koneman's Colour Atlas and Text Book of Diagnostic Microbiology, pp. 726–33.
8. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's Diagnostic Microbiology. 12th edition. Mosby: 2007. Overview of Bacterial Identification Methods and Strategies. pp. 216–47.
9. Facklam RR. Recognition of the Group D Streptococcal species of human origin by doing biochemical and physiological tests. J Appl Microbiol. 1972; 23(6):1131–39.
10. Ruoff LK, Spargo DJ, Ferraro JM. The species identification of the Enterococci isolates from clinical specimens. J Clin Microbiol. 1990; 28:435–37
11. Clinical Laboratory Standards Institute(CLSI).Performance standards for antimicrobial disc susceptibility tests, Approved standards 10th ed.(2010). CLSI document M02-A10, vol 29, No.1.
12. Jain S, Kumar A, Kashyap B, Kaur RI. The clinico-epidemiological profile and the high-level aminoglycoside resistance in enterococcal septicemia at a tertiary care hospital in east Delhi. Int J App Basic Med Res. 2011; 1(2):80–83.
1. Desai PJ, Pandit D, Mathur M, Gogate A. The prevalence, identification and the distribution of various species of enterococci which were isolated from clinical samples, with special reference to the urinary tract infections in catheterized patients. India J Med Microbial. 2001; 19:132–37.
2. Murray BE. The life and the times of the Enterococci. Clin Microbiol Rev. 1990; 3:46–65.
3. Vandamme P, Vercauteran E, Lammens C, Pensart N, Ievan M, Pot B, et al. A survey on the enterococcal susceptibility pattern in Belgium. J Clin Microbial. 1996; 34:2572–76.
4. Ross PW. 14th edition. Elsievier: 2006. Streptococci and Enterococci. Mackie and Mccartney's Practical Medical Microbiology, pp. 268–69.
5. Louie, M., A. E. Simor, S. Szeto, M. Patel, B. Kreiswirth, and D. E. Low et al Susceptibility testing of clinical isolates of Enterococcus faecium and Enterococcus faecalis. J. Clin. Microbiol. 1992;30:41–45.
6. Tritz, D. M., P. C. Iwen, and G. L. Woods, et al Evaluation of MicroScan for Identification of Enterococcus species. J. Clin. Microbiol. 1990;28:1477–1478.
7. Koneman EW, Allen S, Janda WM, Schreckenberger PC, Winn WC. 6th edition. Baltimore Lippincott William and Wilkins; 2006. Isolation and Identification of Streptococci and Streptococci like organisims. Koneman's Colour Atlas and Text Book of Diagnostic Microbiology, pp. 726–33.
8. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's Diagnostic Microbiology. 12th edition. Mosby: 2007. Overview of Bacterial Identification Methods and Strategies. pp. 216–47.
9. Facklam RR. Recognition of the Group D Streptococcal species of human origin by doing biochemical and physiological tests. J Appl Microbiol. 1972; 23(6):1131–39.
10. Ruoff LK, Spargo DJ, Ferraro JM. The species identification of the Enterococci isolates from clinical specimens. J Clin Microbiol. 1990; 28:435–37
11. Clinical Laboratory Standards Institute(CLSI).Performance standards for antimicrobial disc susceptibility tests, Approved standards 10th ed.(2010). CLSI document M02-A10, vol 29, No.1.
12. Jain S, Kumar A, Kashyap B, Kaur RI. The clinico-epidemiological profile and the high-level aminoglycoside resistance in enterococcal septicemia at a tertiary care hospital in east Delhi. Int J App Basic Med Res. 2011; 1(2):80–83.