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Isolation and Characteristion of Rare Gram Positive Cocci in Clinical Samples by Simple, Costeffective, Reproducible Technique.

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ABSTRACT

Gram-positive cocci are among the most frequently isolated bacterial species in clinical diagnostic laboratories. Their identification is currently based on conventional methods, which are time consuming & costly. Hence the present investigation was carried out to screen the different bacterial species from the clinical specimen by Kit method which promises to be a fast, reliable, which will be helpful in effective diagnosis and management of the diseased patient. The differentiation of these species is challenging, our analysis showed the clinical importance of discriminating these species. The study was performed with 100 gram positive cocci from different clinical specimen. They were identified by standard microbiological technique and characterized by the novel, simple, cost effective method which was reproducible, less time consuming and less man power involved.Among the 100 isolates, 50 were identified as Staphylococcus species and the remaining 50 were Streptococcus species. It included 32 Staphylococcus aureus & the remaining 18 were identified by the kit & conventional methods .The other 50 was considered from Streptococcus isolates ,of which 39 were identified as Streptococcus pyogenes & rest of 11 were identified by the kit & conventional methods. Staphylococcus had the following species: *S. carnosus, S. gallinarum* and *S. hyicus, S. schleiferi, S. chromogenes, S. hemolyticus, S.hyicus* and streptococcus included: *Strep.intermedius, Strep.constellatus, Strep.oralis, Strep. acidominimus*. These were found to be rare and unusual species. The use of simple, rapid & cost effective technique proved to be a reliable procedure for & characterization of the gram positive cocci in clinical specimens. It requires only overnight incubation unlike 48hrs required in conventional methods.29 isolates were identified rapidly by this Kit,up to species level. It is reproducible ,less man power involved.There were 6 Isolates which was reported for the FIRST time in India. Incidence of rare species of GPC from clinical sources and their proper identification to the species level will be necessary for management and prevention of these bacteria in any health care facility.

Keywords: Gram positive cocci, Rare isolates, Cost effective, Easily reproducible, First time in India.

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INTRODUCTION

The gram positive cocci are widespread in nature, can be recovered from the environment or as a commensal of the skin, mucous membrane and as pathogen from the infected site. The most important and commonest human pathogens among the gram positive cocci are Staphylococcus and Streptococcus including Enterococci.

They exist as residents in the host [1]. Humans are the natural reservoir for these organisms [2]. They are increasing in isolation & species numbers, as well as in their isolation from the human infections [3]. The finding of 'Resistance' to multiple antimicrobial agents further makes it imperative that the clinical microbiologist should be familiar with isolation and characterization of these organisms.

Therefore we undertook a study of 100 isolates of the gram positive cocci obtained from the clinical samples of the patients attending our hospital, which is a tertiary care centre, situated in suburban Chennai.

The isolates were characterized up to the species level by a novel, simple, cost effective, reproducible technique with high degree of sensitivity and specificity. It also required less man power and time.

MATERIALS AND METHODS

We collected 50 Staphylococci and 50 Streptococci isolates that grew from the different clinical samples of the patients over a period of 3 months (Dec 2013- Jan 2014) visiting our hospital.

The samples were routinely inoculated in Mac conkey agar, Blood agar, Chocolate agar,Bile esculine agar.

The latter two plates were incubated by placing them in candle jar at 37 °C, all the plates were incubated for overnight incubation. The colony morphology was read the next morning. (Images 1,2&3).

The specific colony morphology of ß hemolysis in BA was taken into consideration with supportive positive reactions like: catalase & Tube Coagulase tests for Staphylococci [4] & GROUPING for Streptococci- along with Gram smear for both genera.

These 100 isolates were further characterized up to the species level by the novel, simple, cost effective method & conventional biochemical reactions as per NCCLS guidelines [5]. The organism that has to be identified was suspended in the sterile saline and the turbidity was made to match with Mac Farland 5. As per NCCLS guidelines [5].

Kit components were brought to the room temperature before testing.Simultaneous performance of the conventional procedure was also done for comparative analysis.

For the Staphylococcus the following biochemical reactions such as Voges proskauer, Alkaline phosphatase, ONPG, Urease detection, Arginine utilization, Mannitol utilization, Sucrose utilization, Lactose utilization, Arabinose utilization, Raffinose utilization, Trehalose utilization and Maltose utilization were done,as per the manual given in the kit.

Likewise for the Streptococcus the biochemical reactions such as Voges proskauer, Esculin hydrolysis, PYR, ONPG, Arginine utilization, Glucose utilization, Ribose utilization, Arabinose utilization, Sucrose utilization, Sorbitol utilization, Mannitol utilization and Raffinose utilization were performed, as per the procedure given in the kit.

100 µl of the standardized preparation was inoculated into each vial of the kit. The biochemical reactions were interpreted after overnight incubation which helped us in identifying bacteria up to species level and issue the report with full characterization along with Antibiotic Sensitivity Test after 48 hrs of receiving clinical sample.

Lab standard procedures were followed for interpretation of the conventional biochemical reactions.

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RESULTS

Staphylococcus species

Table 1: The species isolation of staphylococcus and its distribution in relation to different clinical samples.

CLINICAL SAMPLE	TOTAL NUMBER (n=50)	PERCENTAGE
Pus	18	36%
High vaginal swab (HVS)	8	16%
Urine culture	11	22%
Sputum	6	12%
Throat swab	2	4%
Blood culture	3	6%
Eye swab	1	2%
DTT	1	2%

Table 2: Staphylococcus species distribution

SPECIES	TOTAL NUMBER (n=50)	PERCENTAGE
S. aureus	26	56%
S. epidermidis	6	12%
S. carnosus	5	10%
S. simulans	4	8%
S. hemolyticus	3	6%
S. hyicus	2	4%
S. gallinarum	2	4%
S. schleiferi	1	2%
S. chromogenes	1	2%

Streptococcus Species

Table 3: The species isolation of streptococcus and its distribution in relation to different clinical samples.

CLINICAL SAMPLE	TOTAL NUMBER (n=50)	PERCENTAGE
Sputum	13	26%
Pus	10	20%
Urine	11	22%
Throat swab	8	16%
High Vaginal Swab (HVS)	**7**	14%
Blood	1	2%

Table 4: Streptococcus species distribution

SPECIES	TOTAL NUMBER (n=50)	PERCENTAGE
Strep.pyogenes	18	36%
E. faecalis	10	20%
E. faecium	5	10%
Strep. pneumonia	5	10%
Strep.acidominimus	3	6%
Strep. anginosus	2	4%
Strep. sanguinis	2	4%
Strep. oralis	1	2%
Strep. intermedius	1	2%
Strep.canis	1	2%
Strep. constellatus	1	2%

The Gram positive cocci were associated with several clinical infections like wound infections, abscess formation, respiratory infection,bactremia as indicated in table 1 & 3.

The species identification was more rapid by the KIT we used in our study as the species were identified after overnight incubation as shown in table 2 &4.

It was more cumbersome & time consuming by the conventional ,existing lab procedures.

DISCUSSION

By this study we came across not only the usually reported species of *Staphylococcus* such as *Staphylococcus aureus, S. epidermidis* but also unusual species like *S. gallinarum*, *S.carnosus, S. chromogenes. S.simulans* and *S.hyicus*. Similarly In case of streptococcus also we have reported not only the common species such as *Streptococcus pyogenes*, *Streptococcus agalactiae* but also unusual species like *Streptococcus sanguinis, Streptococcus intermedius*, *Streptococcus acidominimus, Streptococcus constellatus.*

This opens the diagnostic arena to explore the rarely encountered species among human infections, which widens and expands the knowledge of clinical microbiologists to be aware of these newly identified pathogens which were seen as veterinary agents [6,7].

The cumbersome preparation of different media and reagents which requires more man power and investment of money in procuring them is overcome by this package which includes all the essential and required parameters for the definite identification and conclusion of species which is of utmost importance for epidemiological and treatment purposes which is highly relevant in patient care & further management.

All these reactions can be interpreted with overnight incubation in contrast to the conventional incubation period of some parameter which has to be observed after 48- 72 hrs of incubation ,example: voges proskauer.

So the entire profile of the bacteria including the species level along with antibiogram pattern is ready within 48 hours of receiving clinical sample, without any further delay. This will ensure the clinician's confidence on the clinical microbiologist & will further improve the initiation of the patient's therapy without any delay, thereby improving the patient's compliance and his economic status. By avoiding the agony of prolonged stay in the hospital & spending more money.

The commonest isolates which we got were *Staphylococcus aureus*- 52%, *Staphylococcus epidermidis* -12%, *Streptococcus pyogenes*- 36%, *Streptococcus pneumonia*- 10%, *Enterococcus faecalis*- 20%, and *Enterococcus faecium*- 20% [8-12].

Among Staphylococcus as well as Streptococcus, the commonly isolated species contributed to 71%. The remaining 29% of the isolates were rare and unusual species which cannot be ignored by a prudent clinical microbiologist because of yester year's commensals are becoming current clinical pathogens [13,14]. So these unreported unidentified rare species has to be brought to the lime light which may be potential human pathogen. So a need for rapid identification and reporting becomes mandate without undue delay.

Hence it requires some other easily reproducible, cost effective technique which was adopted in our study, which aided us in making a reliable and comfortable species identification of the bacteria.

Though some of the rare isolates like *Strep.acidominimus* have been reported from other countries including (CHINA in JAN 2014 [15]). Our study included the FIRST isolation of *Staphylococcus carnosus*, *Staphylococcus hyicus*, *Staphylococcus schleiferi, Staphylococcus chromogenes, Streptococcus constellatus*, and *Streptococcus acidominimus* in INDIA from the patient's samples.

This is to prove that this kit technique is useful in identifying not only usual pathogens but also the rarely encountered & unusual bacteria in association with human infection.

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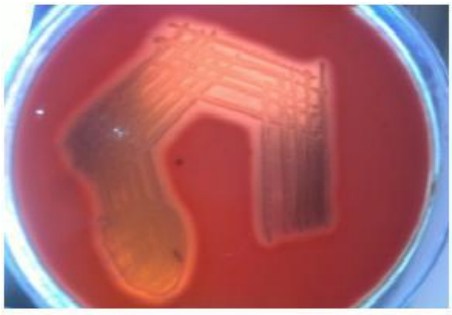
Bile esculine

Biochemical reactions of staphylococcus

Biochemical reactions of streptococcus



Blood agar- hemolysis

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CONCLUSION

It is a gentle reminder for the entire clinical microbiologist to identify the clinical pathogen up to species level for the effective management of the diseased patient by a cost effective, less cumbersome parameter as proved by our study. The procedure can be performed in any given lab & by trained personnel.

Not only it helps in identification but also throws a light on the newer evolving pathogens, which may precipitate life threatening situation if left undiagnosed and undetected. This unfurls the darkness on the unidentified pathogen & paves a path for successful correlation & merging of clinical & diagnostic medicine

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