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KOH String test and Vancomycin Sensitivity Test Resolves the Ambiguity of Gram staining Reaction.

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ABSTRACT

200 bacterial organisms isolated from varied clinical samples received in the central Laboratory. Routine Gram staining was done for all these isolates. In addition to the Gram Staining, all isolates were subjected to Potassium Hydroxide (KOH) String test and Vancomycin Susceptibility Test. Gram negative bacilli showed 100% resistant to Vancomycin and 100% Positive for string test. Gram positive bacteria showed 98% sensitive to vancomycin and 100% negative for string test. Since the test results is very close to the conventional Gram staining reaction, they can be used to supplement the gram staining reaction, in case if there is ambiguity of differentiation.

Keywords: KOH string test, Vancomycin, Gram Staining, Gram negative bacilli, Gram positive cocci

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INTRODUCTION

Time since the advent of Gram staining reaction by Hans Christian Gram, Gram staining is the elementary and the Gold standard test to differentiate Gram positive organism from Gram negative organism. Despite its high specificity, this technique has its own lacunae, resulting in the ambiguity of differentiation of Gram positive from Gram negative organism. The most critical step in the Gram staining reaction is decolourisation[1]. Over-decolourisation or under-decolourisation grossly affect the results, resulting in Gram positive appearing as Gram negative organism and vice versa. In this study KOH string test and Vancomycin susceptibility test are used to differentiate bacterial isolates and the results are compared with conventional Gram staining technique.

MATERIALS AND METHODS

All bacterial isolates are obtained from specimen received at central laboratory of Sree Balaji Medical college chromepet Chennai. All this bacterial isolates are subjected to Gram staining reaction followed by KOH string test and Vancomycin susceptibility test.

Gram Staining

Heat fixed smears prepared from bacterial colony are flooded with crystal violet and allow it to act for one minute and gently rinse with tap water. Flood the slide with Grams iodine and allow it to act for one minute and gently rinse with tap water. Flood the slide with acetone for decolourisation and wash immediately with tap water. Flood the slide with carbol fuschin and allow it to act for one minute and gently wash it with tap water. After drying, the slides are examined under oil immersion (100X) for Gram reaction.

KOH String test

The KOH String Test is done using a drop of 3% potassium hydroxide on a glass slide. A visible loopful of cells from a single, well-isolated colony is mixed into the drop. The suspension was stirred continuously for 60 seconds after which the loop was gently pulled from the suspension. The test was considered positive if the string occurred first 30 seconds after mixing the bacteria in KOH solution.

Vancomycin Susceptibility test

As per the CLSI guideline a standard inoculum of the bacterial colony was prepared and lawn culture was made using Mueller Hinton agar[2]. Vancomycin discs (5µg) were placed on the lawn culture and plates were incubated at 37 °C overnight. Any zone of inhibition is considered as negative.

RESULTS

Out of the 200 isolate 60(30%) isolates were gram positive cocci and remaining 140(70%) were Gram negative rods. Gram positive included Staphylococcus species(Sp), Streptococci sp and Enterococci sp and Gram negative rods included Esch.coli, Klebsiella sp, Pseudomonas sp and proteus species. Results of KOH string test and Vancomycin susceptibility is given in table 1

Table 1

Isolates	KOH String Test		Vancomycin Susceptibility Test	
	Positive	Negative	Sensitivity	Resistant
GPC	0 (0%)	60 (100%)	59 (98%)	1 (2%)
GNB	140 (100%)	0 (0%)	0 (0%)	140 (100%)



DISCUSSION

Gram staining is the first and usually the only method employed for the diagnostic identification of bacteria in clinical specimens. In the current study 59(98%) gram positive cocci were susceptible to Vancomycin and 01(2%) were resistant. The resistant stain was Enterococcus species. In the study by Arthi et al in 2003 all Gram positive species showed 100% sensitivity to Vancomycin.

In contrast to the gram positive organism all Gram Negative organism showed resistance to Gram negative organism and tested positive with KOH string test.

RESULTS

To overcome the ambiguity of Gram staining reaction several tests have been introduced like, demonstration of aminopeptidase enzyme[5] and disruption of Gram negative bacteria cell wall by exposing to alkali[6].The test results of KOH string test and vancomycin susceptibility test closely corresponds to the results of Gram staining reaction and hence this tests can be use to supplement the Gram staining reaction if any ambiguity arises.

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