Mannitol-fermenting and Tube Coagulase-negative Staphylococcal Isolates: Unraveling the Diagnostic Dilemma

Sir,

The identification of bacterial pathogens in human infection plays an important role in the management of patients in health-care institutions. *Staphylococcus aureus* is a ubiquitous commensal bacterium on human skins and anterior nares, but frequently causes severe infections in humans.[1] In developing countries, phenotypic tests are the mainstay in the diagnosis of staphylococcal infections, in which tube coagulase tests (TCTs) are usually confirmatory for *S. aureus*.[2] Although these tests efficiently identify *S. aureus*, their performances vary from setting to setting and need improvement.[3] To achieve presumptive isolation in a single step, mannitol salt agar (MSA) was developed in the year 1945 for the selective isolation of pathogenic staphylococci from the clinical samples.[4] The growth and production of yellow colonies due to the high salt content of media and fermentation of mannitol is regarded as a presumptive tool for the identification of *S. aureus*. It is also described as a characteristic for the differentiation of coagulase-positive staphylococci from coagulase-negative staphylococci (CoNS). However, there are reports that some CoNS can also produce yellow colonies on MSA.[5] The aim of the study was to identify those staphylococcal isolates which were presumed to be *S. aureus* as they produced yellow colonies on MSA but were TCT-negative.

It was a prospective study conducted in a tertiary care pediatric hospital for 3 months from November 2015 to January 2016. A total of 410 isolates of Gram-positive, catalase-positive cocci occurring in clusters were subjected for further identification using MSA and TCT. Human plasma with a dilution of 1:6 was used for TCT. *Staphylococcus* ATCC strains 25923 were used as quality control for both the tests. The isolates that showed yellow colonies on MSA and were negative for tube coagulase ($n = 49$) were further identified by Vitek 2 compact system (bioMerieux, France) compact system.

Of the 49 isolates tested, 24 were isolated from blood, 10 from CSF, 14 from pus, and 1 was from urine sample. Of the 49 isolates tested by Vitek 2C, the maximum isolates were found to be *Staphylococcus hemolyticus* (28.5%), followed by *Staphylococcus xylosus* (26.5%) and *S. aureus* (20.4%) [Table 1].

We evaluated the performance of MSA to identify the *S. aureus* among the tube coagulase-negative *Staphylococcus* isolates. Previous investigations have indicated that 40–50% of the mannitol salt-positive isolates on oxacillin resistance screening agar were, in fact, CoNS.[6,7] Becker *et al.* concluded that the most common mannitol-fermenting isolates were *S. aureus* (48.9%) followed by *S. hemolyticus* (46.2%), *Staphylococcus simulans,* and *Staphylococcus warneri*. Similarly, Blanc *et al.* found that 47% of the isolates were *S. aureus* and 40% were CoNS. In our study, a total of 14 isolates of *Staphylococcus haemolyticus* and 13 isolates of *S. xylosus* utilized mannitol, producing yellow colonies on MSA, with a positive predictive value of 28.5% and 26.5%, respectively. Of the 49 MSA-positive strains which were also tube coagulase-negative, 10 were found to be *S. aureus,* with a positive predictive value of 20.4%. In our study, positive predictive values were much lower as compared to the previous studies. This may be explained by the reason that most of the specimens included in those studies were from anterior nares, throat, and soft tissue, where the isolation rate of *Staphylococcus* species is high, whereas in our study, most of the isolates were taken from blood.

Table 1: Species identification of mannitol salt agar-positive isolates and tube coagulase-negative isolates (*n*=49)

Species	Number of positive isolates (all specimens) (%)	Blood	CSF	Pus	Urine
Staphylococcus haemolyticus	14 (28.5)	8	1	4	1
Staphylococcus xylosus	13 (26.5)	7	6	-	-
Staphylococcus aureus	10 (20.4)	1	-	9	-
Staphylococcus arlettae	3 (6.1)	3	-	-	-
Staphylococcus cohnii	2 (4.0)	2	-	-	-
Staphylococcus hominis	1 (2.0)	1	-	-	-
Staphylococcus saprophyticus	1 (2.0)	1	-	-	-
Staphylococcus sciuri	1 (2.0)	-	1	-	-
Staphylococcus warneri	1 (2.0)	-	1	-	-
Staphylococcus equorum	1 (2.0)	1	-	-	-
Unidentified	2 (4.0)	-	1	1	-
Total	49	24	10	14	1

Second, we included only those strains of *Staphylococcus* species which were negative for TCT. Two strains were not identified by Vitek 2C system, the reason for which may be attributed to the unavailability of data for possible *Staphylococcus* species in its database.

The coagulase-negative *S. aureus* may probably be MRSA isolates, which are reported to react weakly or negatively with TCTs,[8] or rare *S. aureus* strains that are reported to be coagulase-negative.[9] Another reason for *S. aureus* for being tube coagulase-negative can be due to the usage of human plasma because rabbit plasma was unavailable. When using human plasma, it is advisable to pool together samples from at least 5–10 persons and then distribute them in small aliquots sufficient to last for 1 week and freeze the rest until required. This will help eliminate errors due to insufficient fibrinogen or the presence of inhibitory substances in any particular patient's sample.[2]

The identification of clinical *S. aureus* is largely performed by TCT, but it requires screening of the isolates with MSA prior to TCTs, for improved efficiency. MSA alone cannot be used for the identification of *S. aureus*. There is no single phenotypic test (including the TCT) that can provide reliable results in the identification of *S. aureus*, and a combination of tests should be used for the correct identification of isolates.

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Conflicts of interest

There are no conflicts of interest.

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