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Screening for Methicillin resistant Staphylococcus aureus (MRSA)

This document describes laboratory methods of screening for MRSA carriage. It does not include recommendations on indications for screening, sampling methods or equipment used. Infection control measures for established MRSA carriage should be determined by national public health authorities.

Introduction

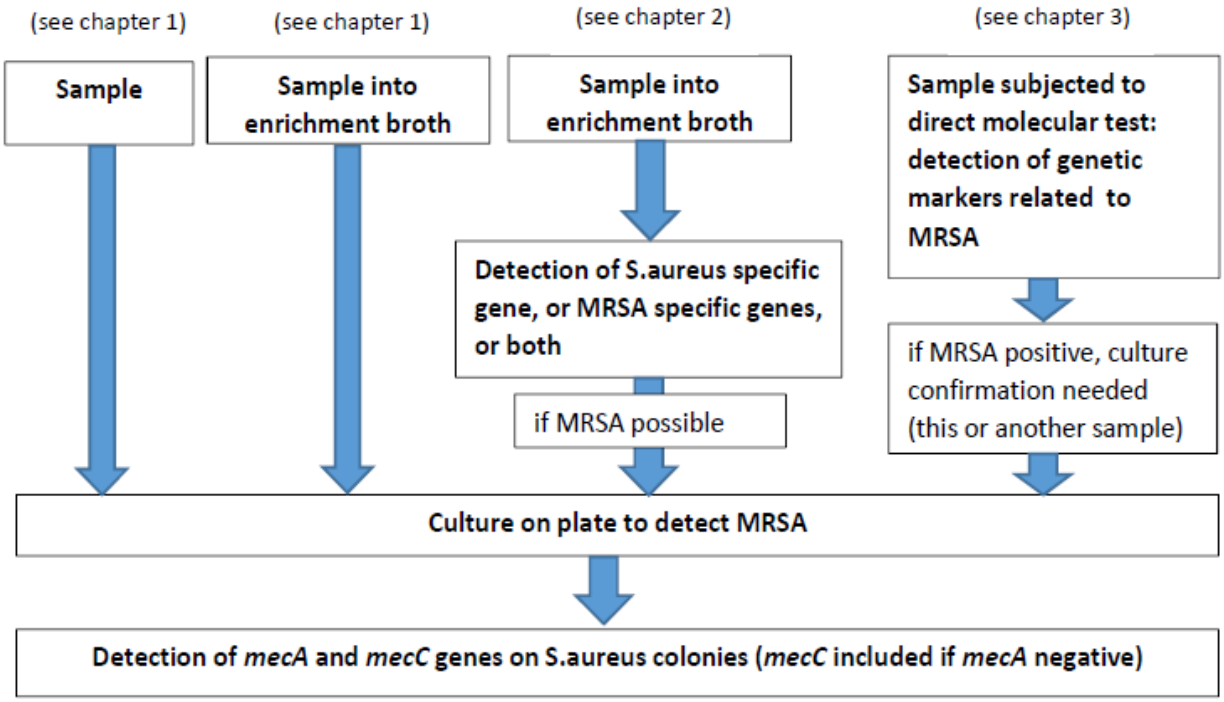
Detection of MRSA carriage is an important component of strategies for preventing the spread of MRSA. The quantity of Staphylococcus aureus in a sample can be very low and other bacteria can be present in a significantly higher quantity. Several studies show that the use of enrichment broth culture increases the diagnostic sensitivity for detection of MRSA by 15-20% in single set of screening samples (1,2,3,4), but it is not clear how enrichment broth effects sensitivity when multiple sample sets are taken. The recommendation for using enrichment broth should follow local/regional/national guidelines. The presence of MRSA in a sample or in enrichment broth is detected by culture based methods and/or by detection of genomic targets.

Final confirmation of MRSA carriage requires culture, followed by detection of *mecA*/*mecC* genes from *Staphylococcus aureus* colonies.

In Nordic countries the most commonly sampled anatomical sites include nose, throat and perineum, although sampling sites may vary between different situations, organizations and countries. Pooling samples taken from several anatomical sites of one patient into a single transport or enrichment media is often done to reduce cost, but may to some extent reduce the sensitivity of screening (5). If samples are pooled it is not recommended to include fecal/rectal samples and the process should ideally be validated to assess for reduction of sensitivity when pooling. If only pooled samples are analyzed, it will not be clear which anatomical sites are positive or negative.

Laboratory screening strategies

There are several possible approaches to MRSA screening within the laboratory. The trade-off between sensitivity and specificity varies between strategies for screening. The strategy chosen should serve the local circumstances and context for screening, for example, to produce quick negative results if needed, to ensure optimal sensitivity, or to adjust the process to the amount of screening samples. Molecular tests with variable genetic targets (*S. aureus* specific genes/*mecA* and *mecC* genes/gene sequences located at SCC*mec* junction) are widely available commercially.



Description of the methods

1. Culture-based detection of MRSA

Culture-based MRSA screening can produce a negative result in 24-48 hours (if samples are cultured on a plate) or in 48 hours (if samples are enriched in broth and then cultured on a plate).

If enrichment is used, the sample is transferred into selective enrichment broth (6,7,8,9,10,21,22), for example with 3,5 mg/L cefoxitin and 20 mg/L aztreonam as selective agents (21). If enrichment broth containing salt (NaCl) is used, it is important to note that higher concentrations which are more selective (e.g. 6.5-7.5% NaCl) inhibit the growth of some strains of MRSA. Therefore, a lower concentration of 2.5% NaCl may be preferable for increased sensitivity (19). The broth should be incubated at 35-37^{o}C in ambient air for a minimum of 8 hours, preferably longer (overnight).

A sample or an aliquot of incubated enrichment broth is plated on a selective MRSA chromogenic agar plate (20), and/or on a 5% blood agar plate with cefoxitin disk/tablet, or on a chromogenic SA plate with cefoxitin disk/tablet. The plate is incubated at 35-37^{o}C in ambient air for 24-48h, following manufacturer instructions if a chromogenic plate/agar base is used. However, rare CO_2 dependent isolates will not be detected when using plates incubated in ambient air.

Colonies identified or suspected to be *Staphylococcus aureus* are subjected to a molecular confirmation test to detect *mecA* and *mecC* genes. If the test used detects only *mecA*, detection of *mecC* should be performed on all *mecA* negative strains. Species identification of *mecA*/*mecC* positive isolates is essential: if the molecular *mecA*/*mecC* detection method does not contain a *S. aureus* specific target, then species identification must be performed by another method.

Laboratories without timely access to molecular confirmation tests may use a PBP2a agglutination test for initial confirmation of MRSA. However, these tests usually only detect *mecA*-encoded PBP2a and may not be as reliable, so final confirmation of MRSA requires molecular detection of *mecA*/*mecC* genes.

2. Molecular detection of MRSA from enrichment broth

Molecular detection of MRSA from enrichment broth is suitable for settings where negative screening result should be

achieved in approximately 24h and a high proportion of negative samples is expected. If so, enrichment broth from up to three samples from the same patient can be pooled for molecular testing. If fecal/rectal samples are taken, pooling with other samples is not recommended.

An aliquot of enrichment broth is subjected to a molecular test to detect *S. aureus* specific targets (e.g. *nuc*, *co*A, *Sa*442 o *spa*) or *mecA* and *mecC* genes in parallel with a *S.aureus* specific target (7,11). Also, additional MRSA specific molecular tests targeting the chromosomal integration site of the SCC*mec* may be used (12). Samples with a negative molecular test result (i.e. negative for *mecA*/*mecC* genes and/or a *S. aureus* specific gene) are confirmed negative screening samples. Samples with a positive signal for *S. aureus* or MRSA specific targets are cultured on plate as described previously (chapter 1).

It should be kept in mind that a false negative result in a molecular test is possible if one or several of the genomic targets are altered in the genome of the MRSA strain in question. However, for *S. aureus* specific genes this is very rare. Nonetheless, awareness of the divergent features of the circulating strains is important and the capability of the diagnostic tests to detect different variants should be continuously updated. In case of suspected or confirmed defects in the molecular method, alternative methods should be used.

3. Molecular detection of MRSA direct from screening samples

Molecular detection of MRSA specific targets offers the fastest way to produce a negative or a preliminary positive screening result from a sample. This can be achieved within a couple of hours. There are numerous commercial tests available, most often validated for nasal swabs only (13,14,15,16).

To produce a specific positive MRSA screening result, the molecular test should be able to confirm that the detected *mecA/mecC* gene is located within the *Staphylococcus aureus* genome, usually by targeting the chromosomal integration site of SCC*mec*.

It should be emphasized that these tests will not achieve 100% sensitivity or specificity (17,18). Tests targeting only *S. aureus* specific genes together with *mecA*/*mecC* genes may produce false positive results, if the sample contains *mec* negative *S. aureus* together with staphylococcal species other than *S. aureus* carrying a *mec* gene. Also, false positive results can occur if the *S.aureus* isolate contains an empty SCC*mec* cassette (cassette with *mecA*/*mecC* gene deleted). As with molecular tests in general, false negative results may occur for genetic variants with altered target sequences or samples with very low concentration of the target.

Regardless of which genetic targets are detected by the test, the positive result should be taken as preliminary. The final confirmation of MRSA carriage must be verified by culture with detection of *mecA*/*mecC* gene from *S.aureus* colonies as described previously (chapter 1).

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