Summary and Conclusion

200 isolates were identified from 220 clinical samples collected from Benha university hospital to isolate any growth of Staphylococcus aureus .

The age of the patients ranged from 3 days to 55 years with the mean age 27.5years.

The sex of the patients under the study was 65 female and 155 male.

These samples were cultured on (blood agar, Mac-Conkey and mannitol salt agar).

The 200 isolates were collected from pus (128), ear discharge (2), urine (43) and blood (27) .

- It was shown that the prevalence of gram positive cocci was 41.5% (83 Gram+ve cocci out of 200 isolates).
- The prevalence of Staphylococcus aureus isolates was 28% (56 S.aureus isolates out of 200 isolates).
- The prevalence of other gram positive cocci was 13.5% (27 out of 200 isolates), 25 of them were coagulase negative staphylococci and 2 were streptococci.
- The prevalence of gram negative bacilli was 58.5% (117 out of 200).

Identification of MRSA was done by the following methods:

Detection of mec A gene(the gold standard method) was done by real time PCR(22 S.aureus strains were MRSA and 34 S.aureus were MSSA).

Phenotypic identification was done and compared with real time PCR and it was found that:

- Disk diffusion method :

By oxacillin 1ug, the sensitivity of this method was 90.9% and specificity was 94.1%.

By oxacillin 5ug, the sensitivity of this method was 90.1% and specificity was 76.5%.

By cefoxitin 30 ug, the sensitivity of this method was 100% and specificity was 94.1%,

- E-test MIC strips with oxacillin , the sensitivity of this method was 90.9% and specificity was 94.1%.
- The PBP2a latex agglutination test, the sensitivity of this method was 100% and specificity was 100%.

It is found that real time PCR and latex agglutination test did not detect two border line resistance S.aureus (their MIC was 4-8ug/ml) as they did not have mec A gene and also lack PBP2a (the product of the gene).These BORSA strains was detected by all used disk diffusion methods and E-test.

From our results, it is declared that if compared with real –time PCR only latex agglutination test is able rapidly and reliably to detect methicillin resistance in S.aureus as it yielded the best sensitivity and specificity.

The time taken by latex agglutination test for detection of MRSA from isolated S.aureus colonies was 20 min. The PBP2a latex agglutination assay is less complicated than PCR for mec A and has been shown to be

more sensitive than other phenotypic methods, such as the use of oxacillin disk diffusion method and oxacillin E-test strips.

However, cefoxitin 30ug disk diffusion test yielded the sensitivity of latex agglutination test (100% for both of them) but its specificity was lower than latex and it can be used as screening test as it gave no false negative results, however it is not a conclusive test as it detects 2 hyperproducers of B-lactamases as MRSA and it is also time consuming test.

Oxacillin E-test strips yielded the same sensitivity and specificity of the oxacillin 1ug disk diffusion method.

In conclusion, the new molecular assay (real time PCR) was found to be rapid and robust. Because it is a largely automated assay, less hands-on work is needed, consumes shorter time than conventional PCR and it can be used for direct detection of MRSA from non sterile clinical specimen, however, it is not yet available in the majority of routine diagnostic laboratories because of their elevated technical requirements.

In absence of real time PCR, latex agglutination test is the best method for MRSA detection from isolated colonies.

Both real time PCR and latex agglutination test avoid missing of cryptic MRSA strains which should be treated with vancomcin and other antimicrobials against MRSA. Also avoid unnecessary use of vancomycin to B-lactamase hyper producers (BORSA) strains which could be treated with B-lactam –B-lactamase inhibitor compounds.