toothbrushing habit varied from 42.6% in male and 27.6% female in intermediate to 61.4% male and 26.8% female in secondary schools.

Miswak was used more than 3 times a day by 53% male and 24% female student at intermediate schools, while this increased to 56% in male and decreased to 17% in female at secondary schools. The over all time spent on brushing was less as compared to miswak in average more than 3 minute per use, and it is in agreement with previous study. This shows that more time is being spent on miswak compared to toothbrush but that does not reflect the meaningful cleaning of teeth by miswak as most of the male chew the miswak as a cultural norm. The majority of the students in both groups of schools expressed that toothbrush clean better than miswak, while majority of the miswak user, use it due to Sunnah with second feeling of better cleaning. The combined users of miswak and toothbrush ranged 53-68% in expressing the understanding of better cleaning. Recently, Darout et al[5] assessed and compared the periodontal status of adult Sudanese habitual miswak and toothbrush users. It was found that the periodontal status of the miswak users in the Sudanese population was better than that of toothbrush users and the efficacy of miswaks used for oral hygiene was comparable to or slightly better than that of the toothbrush.

All of the above-mentioned comparative studies are in favor of promoting traditional oral hygiene tool of miswak. This can be integrated for oral health promotion activities, and the target group of oral hygiene neglectors should be persuaded to start with miswak, which is socially and culturally accepted and religiously motivated as the finding of present study has highlighted. The other important finding in this study is that female students in secondary school groups have descending trend of oral hygiene practices. As we know that today's children or adolescent are tomorrow's parents, so the female students needed to be focused for effective orientation for toothrushing, as before becoming mothers.

Almost 10% of intermediate school students and 17% from secondary schools never cleaned their teeth. Female students have better oral hygiene practices as compared to male students at intermediate level but trend shifts towards the male students at secondary school level. Toothbrushing was most commonly used by female students at both intermediate and secondary schools. Both groups of students and both groups expressed the common use of toothbrush due to better cleaning perception. Miswak was used by 24% secondary school male students. Majority of the students used old miswak, and unspecific technique was most commonly used. Sunnah was expressed as common motivation of using miswak.

Oral health educational activities at schools should be integrated with oral health promotional approach. Female students at schools should be given more knowledge and incentives to improve and develop oral hygiene practices on regular basis. The parents and teachers should play a major role in promoting healthy oral habits among students. Further, research is needed to evaluate the effectiveness of oral hygiene habits and to compare the oral health status among the studied population.

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Comparison of PCR and disc diffusion methods in detecting methicillin resistance among *Staphylococcus species* from nosocomial infections

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Methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin resistant coagulase negative *Staphylococcus species* (MRCON) have spread worldwide after the introduction of methicillin. Methicillin resistant *Staphylococcus aureus (S.aureus)* was first isolated in England in 1961 shortly after the introduction of methicillin. Resistance *Staphylococcus species* are an important cause of nosocomial and community infections. Nowadays, there is an increase in the prevalence of MRSA and MRCONS worldwide. This increase requires a rapid, accurate and sensitive method for isolation methicillin resistant isolates. Methicillin resistant *Staphylococcus species* are

Table 1 - Comparison of methicillin resistant *Staphylococcus aureus* and methicillin resistant coagulase negative *Staphylococcus species* by PCR with disc diffusion method.

Method	PCR Resistant strains	PCR Sensitive strains	Total	Reliability
MRSA	20	4	**24**	Sensitivity=76.9%
MSSA	6	72	**78**	Specificity=94.7%
	26	76	**102**	Accuracy
MRCON	21	1	**22**	Sensitivity=87.5%
MSCON	3	71	**74**	Specificity=98.6%
	24	72	**96**	Accuracy=93.1%

MRSA - methicillin resistant Staphylococus aureus,
MSSA - methillin sensitive Staphylococcus aureus,
MRCON - methicillin resistant coagulase negative Staphylococcus species,
MSCON - methicillin sensitive coagulase negative Staphylococcus species

resistant practically to all lactam antibiotics that are represented by penicillin and cephalosporin. It is difficult to detect all methicillin resistance by routine susceptibility methods as of the heterogeneous nature of methicillin resistance.[1] Genetically, methicillin resistance determined by a chromosomal gene (meca), which has been cloned and sequenced from a Japanese MRSA mec A gene codes for a penicillin binding protein (PBP2a). Acquiring PBP2a[2] by *Staphylococci species* convert them into resistant strains. Meca gene detected in methicillin resistant and absent in methicillin sensitive *Staphylococci*, so it is a useful molecular marker to differentiate between methicillin resistant and sensitive *Staphylococci species*. Evaluation of disc diffusion assay in detecting methicillin susceptibility Staphylococcus was controversial. Prasad et al[1] considered disc diffusion the least reliable assay (87.7% sensitivity and 89.8% specificity) compared with microdilution and PCR assays for detection of methicillin resistance *Staphylococcus aureus*. Other reports[3] showed that disc assay are reliable and has a positive predictive value 95% for coagulase negative *Staphylococcus species* and could detect over 94%of meca positive isolates. Many previous reports used PCR assay for the detection of methicillin resistance through amplification of meca gene and considered as a gold standard for detecting methicillin susceptibility.[2] The aim of this study is to compare sensitivity, specificity and accuracy of disc diffusion with meca amplification PCR assay for detecting methicillin resistance *S.aureus* and coagulase negative *Staphylococcus species*.

Bacterial strains. One hundred and two *S.aureus* strains and 96 coagulase negative *Staphylococcus species* were collected from different clinical samples from Prince's Basma Hospital in Irbid. *Staphylococcus aureus* (ATCC 25923) was used as a control. Staphylococcus isolates were classified into 4 groups: MRSA, MSSA, MRCON and methicillin sensitive coagulase negative *Staphylococcus species*. Upon receipt of clinical samples, they were subcultured on blood agar plates and incubated at 37°C overnight. Staphylococcus isolates were identified by biochemical and physiological tests.

Disc diffusion susceptibility test. Disc diffusion test was carried out following the National Committee Clinical Laboratory Standard. The final inoculum was adjusted to 5 x 10^7 to 9 x 10^7 CFU/ml in Mueller-Hinton broth (Difco Laboratories). The inoculum was plated by sterile swab uniformly on Mueller-Hinton agar (Difco laboratories) and 5 µg methicillin discs (Sigma) were applied. Plates were incubated at 25°C for 24 hours.

Oxacillin microdilution. Oxacillin broth susceptibility test was carried out as NCCLS recommendations in Muller-Hinton broth.

Deoxyribonucleic acid extraction method. Deoxyribonucleic acid was extracted form Staphylococcus strains using Wizard kit (Promega) with slight modification. Proteinase K enzyme (20mg/ml) was added with lysostaphin to increase the lysis of Staphylococcus cell wall.

PCR assay. The extracted Staphylococcus DNA samples were used to amplify Staphylococcus meca gene. Amplification of meca gene is a 997bp product. Amplification was performed with the following primers: 5`-CAT TTT GAG TTC TGC ACT ACC 3` and 5` GCA ATA CAA TCG CAC ATA CAT TAA

TAG 3`. Primers, dntps, Taq DNA polymerase, Taq buffer, $MgCl_2$ were obtained from Promega (USA). The thermocycling mix contained 0.5 μg of the extracted DNA 0.5 μM of each primer, 100 μM of each dNTP and one unit of Taq DNA polymerase, 2.5μM $MgCI_2$. The thermocycling conditions as follows: 95°C for 5 minutes for one cycle then 95°C for one minute, 57°C for one minute and 70°C for 2 minutes for 35 cycles on thermalcycler (Biorad, USA). The reaction then incubated at 72°C for 10 minutes. Ten microliters were removed from the amplified mixture and subjected to ethidium bromide agarose gel electrophoresis (1.5% agarose) at 70 Volts for one hour along with 100 bp stepladder (Poromega). Agrose gel then stained, visualized under ultra-violet light and photographed by photo-documentation system (Vilber Lourmat).

The presence of meca gene was tested for 102 *Staphylococcus aureus* and 96 coagulase negative Staphylococcus disc diffusion fails to detect 6/26 MRSA and 3/24 MRCON. This test also identifies 4/76 MSSA as MRSA and 1/72 MSCON as MRCON. The sensitivity, specificity and accuracy of disc diffusion was 76.9%, 94.7% and 85.8% for *Staphylococcus aureus* and 87.5%, 98.6% and 93.1% for coagulase negative Staphylococcus **(Table 1)**. The discrepant MSSA (6/26) and MSCON (3/24) were retested by broth microdilution assay. The results showed that 4/6 MSSA and 2/3 MSCON had minimum inhibitory concentrations (MIC) of more than 8μg/ml for methicillin and therefore had posses the methicillin resistance gene. The other 3 were meca negative and had their MIC 0.5μg/ml indicating sensitivity to methicillin. The other discrepant MRSA 4/76 and MRCON 1/72 strains were also retested by microdilution assay and their MIC to methicillin were 4 and 8 μg/ml (borderline resistant). They were also retested for amplified meca gene and showed PCR product (997bp) upon retesting.

Methicillin resistant Staphylococci are one of the most common causes of nosocomial infections. Standard bacterial identifications and susceptibility testing frequently require as long as 72 hours and there may be a difficulty in identifying methicillin resistance due to the heterogeneous nature of resistance to methicillin. Disc diffusion fails to detect 6 MRSA and 3 MRCON while their resistance was detected by PCR assay through meca gene amplification. Microdilution in Muller-Hinton broth for these 9 discordant strains showed that 4/6 and 2/3 were methicillin resistant and three were methicillin sensitive. The last 3 were mecA positive, these may have a nonfunctional meca gene or a nonactive PBP2a protein. This also indicate that disc diffusion assay has the lowest sensitivity compared to microdilution and PCR assay in detecting methicillin resistant. Five mecA negative (4 MRSA and 1 MRCON) were resistant to methicillin by disc diffusion assay. Minimum inhibitory concentrations of microdilution showed that all isolates have a borderline resistance (MIC 4-8 μg/ml). These resistant strains should be retested for possibility of β-lactamase over production 2 or for modification of normal PBP2agene.[4]

This study shows that the molecular assay for mec A by PCR should be considered as a gold standard for detection Staphylococcal methicillin resistance as reported previously.[1,2,4,5] Negative mecA may show methicillin resistance and positive mecA may be sensitive[2,4] so a combination of both molecular and microbiological may be the best for detecting methicillin resistant *Staphylococcus species*.

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Osmotic fragility and Na^+-K^+ ATPase activity of erythrocytes of HIV/AIDS patients

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Symptomatology of human immuno-deficiency virus/acquired immuno-deficiency syndrome (HIV/AIDS) is very diverse. However, anemia is one of the most universal clinical symptoms of the disease. The etiology of anemia in HIV disease has been extensively researched primarily from the