Academic Journal of Nawroz University (AJNU), Vol.11, No.1, 2022

https://doi.org/10.25007/ajnu.v11n1a1315

Title of the article: Molecular Characterization of Sarcoptes scabiei and Methicillin-resistant Staphylococcus aureus among Internally Displaced Children in UNHCR Refugee-IDP Camp.

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ABSTRACT

Scabies is a common dermatological condition, frequently associated with pyoderma especially in warm climatic areas causing the most common dermatological infection worldwide. The indirect health impact of scabies complication, including secondary bacterial infection imposes a major cost on health-care systems particularly among internally displaced children in refugee camps. There is inadequate information concerning the genetic diversity of populations of the scabies mites in humans in Iraq. The objective of this study was to measure the strength of association between pyoderma and scabies in addition to genetic characterization and variation in the DNA sequences of Sarcoptes scabiei and Methicillin-resistant Staphylococcus aureus (MRSA) in internally displaced children in UNHCR Refugee-IDP camp in Zakho, Iraq. Molecular characterization was performed using ITS2 rDNA as a specific marker for targeting the nuclear ribosomal DNA of Sarcoptes scabiei and specific primers mecA for targeting the mecA gene of MRSA using PCR techniques. There are no previous studies in Iraq in this direction. The results revealed a 480 bps PCR product amplified from the DNA of S. scabiei isolates and 310 bps PCR product from the DNA of MRSA, which indicating specific detection of S. scabiei var. hominis and MRSA from infected children. Four isolates of scabies and one isolate of MRSA were subjected to DNA sequence of ITS2 and to partial DNA sequence of mecA gene to access the relation with other global isolates. The obtained sequences were submitted to GenBank with the following accession numbers (MW261329, MW261330, MW261331, MW115895, MW165336). PCR assay has increased sensitivity over microscopic examination especially during outbreak investigation of scabies when large number of suspected patients are screened and untreated scabies is often associated with pyoderma.

KEYWORDS: Displaced children, scabies, pyoderma, MRSA.

1. Introduction

Scabies is a skin condition caused by *Sarcoptis scabiei* mites is more common among people practicing poor hygiene, those living in overcrowded condition, furthermore, it affects both sexes and all ages regardless to their socioeconomic states and hygienic level (Zayyid *et al*., 2010). Scabies can be transmitted by direct skin contact among member of the same family, or through inanimate objects (clothing and furnishings) and also sexual contact is another essential route for transmission among adults (Zayyid *et al*., 2010). The main manifestations of scabies are mediated from the inflammatory response through the stimulation of adaptive immune response to the mite and its products (Roberts *et al*., 2005). Scabies is of four types; Classical scabies presenting as pruritus commonly occur between the fingers, hands, feet and axillary and periumbilical area; Nodular Scabies presents with red brown nodules appeared after months of treatment; Atypical scabies, very rare and uncommon type, but it is common among very young and immune compromised people (Bhat *et al*., 2017). Lastly the Norwegian scabies or Crusted scabies is more common among patients suffering from debilitated immune system due to presence of large burden of mites in the skin producing large number of eggs inside the skin. Crusted scabies is associated with high level of eosinophilia and immunoglobulin (IgE) (Bhat *et al*., 2017).

Scabies is considered as an important risk factor for secondary bacterial infection such as pyoderma caused by *Streptococcus pyogenes* and *Staphylococcus aureus* (Aung *et al*., 2018). Infection with pyoderma may lead

to other severe complications such as sepsis, heart disease and kidney infections (McDonald *et al*., 2007). Although both *S. aureus* and *S. pyogenes* (Group A) are considered as causative agents of pyoderma, but they have different mechanisms of colonization; *S. aureus* firstly colonize nasal epithelium then colonize the skin, whereas, Group A streptococci directly bind to epithelial cells of the skin trauma. MRSA is a multidrug resistant strain of *S. aureus* has a major role in secondary bacterial infection associated with scabies (Swe and Fischer, 2014). Besides that, scabies mites could conquer the immediate host immune response by secreting a serine protease inhibitor (serpin B4/SMSB4) with their faeces into the epidermal mite burrows which consequently inhibits complement system and providing favorable conditions to promote the growth of *S. aureus* and Group A Streptococci (Swe and Fischer, 2014).

Although diagnosis of scabies usually is based on morphological characteristics, PCR is considered as the best diagnostic technique performed for amplification of DNA markers such as ribosomal Internal Transcribed Spacer region (ITS2) for scabies and *mecA* gene for amplification of methicillin resistance *S. aureus*(MRSA) which carry *mecA* gene on their Staphylococcal Chromosomal Cassette (SCCmec) and encoded for Penicillin Binding Protein A (PBPA) which is a transpeptidase enzyme catalyzes cell-wall crosslinking and responsible for methicillin resistance (Rasheed and Hussein, 2020). Due to the in availability of studies on this direction this study was adopted to identify the mite species and the bacteria associated with the produced lesion using ITS-2 and *mec A* gene sequences.

2. Materials and Methods

2.1. Study Samples

This study was conducted during the period from March 2018 to March 2020, during which specimens of skin-scraping and skin swabs were collected from 665 Internally displaced children aged 4-10 years from both sexes (395 boys and 270 girls), living in Cham Mishko camp, Zakho city, Duhok governorate. These patients were suffering from scabies lesions secondary infected. The lesions were manifested by erythema, oozing, vesiculopustular lesions, and pus formation. None of the patients had received prior local therapy with antibacterial, antifungal, or antiscabies agents.

Skin scrapings were taken using sterile disposable scalpel blades and stored in a fully labeled sterile plastic container with tight lids, for skin swabs, a sterilized swab was moistened with sterile normal saline and rolled gently on the skin with impetigo, then placed in a labeled tube containing Brain–Heart Infusion Broth as transporting medium (Neogene, UK) for isolation and identification of *Staphylococcus* spp. All samples were examined within six hours of collection. For the detection and identification of scabies, each skin scrap was mixed with two drops of 10% potassium hydroxide (KOH) and examined under the microscope to detect the mite, their eggs or products (Alasaad *et al*., 2009). Crusted scabies was recognized as the patients showed typical psoriasiform dermatitis with hyperkeratotic and plaques (Stone *et al*., 2008).

2.2. Bacteriological investigation

For detecting the presence of *Staphylococcus* species in skin lesions and pyoderma, all collected swabs were inoculated on 5% sheep blood agar for 24 hours at 37^{o}C. Beta hemolytic colonies were then re-cultured on Mannitol salt agar as selective medium then incubated at 37°C for another 24 hours, the fermented yellow colonies were picked and stained with Gram stain to study their microscopic characteristics (Barrow and Feltham, 2003). Other biochemical tests such as catalase and coagulase test were performed for further conformation (William *et al*., 2003). For the detection and identification of methicillin-resistant *S. aureus*, all isolated colonies were confirmed for oxacillin susceptibility. Isolated colonies were cultured on Mueller–Hinton agar (MHA) (Neogene, UK) amended

with 6 mg/ml of oxacillin. After 24 hours of incubation at 35°C, the zone of inhibition was measures according to guidelines of Clinical and Laboratory Standards Institute (CLSI, 2015).

2.3. Genomic DNA extraction from skin samples

Genomic DNA extraction Kit (Addbio, Korea) was used for the extraction and purification of DNA from hundred skin scraping specimens which considered as positive microscopically. The extraction and purification were performed according to manufacturer's instructions. The concentration and purity of extracted DNA was estimated by Nanodrop (Thermo Fisher Scientific).

2.4. Genomic DNA extraction from bacterial colonies

DNA was extracted from hundred positive bacterial cultures using DNA Mini Kit for extraction and purification of the bacterial DNA (Invitrogen, USA). Yellow colonies on Mannitol Salt Agar were re-cultured into Brain Heart Infusion broth (BHI) and incubated for 18 hours at 37°C. One mL of fresh incubated BHI broth was used for DNA extraction kit. The purification process of extracted DNA was conducted using lysosome and proteinase K in accordance to the kit manufacturer's protocol. The concentration and purity of extracted DNA was estimated by Nanodrop (Thermo Fisher Scientific).

2.5. PCR amplification of *Staphylococcus* genus-specific *mecA* gene

Molecular investigation was performed by amplifying *mecA* gene using one primer pairs (Table 1). The PCR setting for *mecA* gene was as follow; initial denaturation at 94°C for 5 minutes, 30 cycles of denaturation at 94°C for 45 sec, annealing at 60°C for 45 seconds, and extension at 72°C for 90 sec, and a final extension cycle at 72°C for 6 min. The PCR products were electrophorized on 1.5% agarose gel containing Red Safe dye with green fluorescence (GeNet Bio, Korea). DNA ladder with molecular weight 100 – 1000 bps was included (Thermo Fisher Scientific, United States). Gel was viewed under a UV Transilluminator (Maestrogen, Taiwan) to detect the expected band sizes.

2.6. PCR amplification of ITS-2 of *Sarcoptes scabiei*

For the amplification of ITS2 of *Sarcoptes scabiei*, one pair of specific primers were used (Table 1). The PCR setting was: the initial denaturation at 95°C for 2 minutes, 30 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 50 seconds, and a final extension at 72°C for 7 min. The PCR products were electrophorized on 1.5% agarose gel containing Red Safe dye with green fluorescence (GeNet Bio, Korea). DNA ladder with molecular weight 100 – 1000 bps was included (Thermo Fisher Scientific, United States). Gel was viewed under a UV Transilluminator (Maestrogen, Taiwan) to detect the expected band sizes.

Table 1: specific primer for detection of *Staphylococcus aureus* MRSA and Scabies

Genes	Sequence primer	Amplicon Size (bp)	References
*mecA*_F	5'-GTAGAAATGACTGAACGTCCGATAA-3'	310	(McClure *et al.*, 2006)
*mecA*_R	5'- CCAATTCCACATTGTTTCGGTCTAA-3'		
ITS-2_F	5'-CGACTTTCGAACGCATATTGC-3'	480	(Noge *et al.*, 2005)
ITS-2_R	5'-GCTTAAATTCAGGGGGTAATC-3'		

2.7. Sequences analysis and phylogenetic relationships

PCR product of twenty Scabies specimens and twenty-five MRSA isolates were sequenced using ITS2 and *mecA* primers, respectively by Macrogen company (Seoul, South Korea). The obtained DNA sequences were analyzed using BLAST Basic Local Alignment Search Tool (http:// www.ncbinlm.nih.gov/BLAST). BioEdit software was applied for trimming and clean-up of aligned sequences. The obtained nucleotide sequences were submitted to the GenBank to get the accession number. The phylogenetic tree for obtained sequences (accession number) and related sequences

retrieved from the GenBank database was conducted by MEGA10 software using Neighbor-Joining method and aligned using CLUSTAL W multiple alignment program.

3. Results

The microscopical examination; revealed that 47.3% (315/665) of the samples confirmed as typical scabies and two cases of crusted scabies were also diagnosed. Regarding pyoderma, 39.4% (264/665) of the samples were positive after culture verification. Regarding scabies, 96% (96/100) skin scraping samples isolated from internal displaced children, were positive microscopically and proceed for DNA extraction and further confirmation by molecular techniques using PCR for ITS-2, all of them revealed 480 bps on agarose gel electrophoresis by amplification. The amplified sequences of scabies samples have been deposited in GenBank under following accession numbers (MW165336, MW261329, MW261330, MW261331) and showed very similar homology with scabies sequences deposited in GenBank. PCR amplification of *mecA* gene was done to detect *Staphylococcus aureus* Methicillin resistance (MRSA) from hundred positive bacterial cultures. The size of PCR product was 310 bps in accordance to *mecA* gene. All the tested samples were positive for both techniques. The amplified sequence of PCR product has been deposited in GenBank under the accession number (MW115895) and showed very similar homology with MRSA sequences deposited in GenBank. The phylogenetic tree was constructed using the partial genome sequence of obtained accession number of scabies with other related sequence from the GenBank. The phylogenic analyses showed that the isolated *S. scabiei* mites were distributed into two genetically distinct clades and that each clade formed a well-supported monophyletic group (Figure 1).

The first clade includes sequences obtained in Pakistan, Chile and Egypt. While the geographic distribution of the second clade was restricted to Iraq isolated sequences. The phylogenic tree showed that IQ-isolate MW261329 and MW261331 are sister taxa in 72% of bootstrap and IQ-isolate MW261330 and MW165336 are sister taxa in 77% of bootstrap. The result of the alignment showed high identify 98-100% in sequence of Iraq mites as shown in Figure (1). Regarding the MRSA *Staphylococcus aureus*, the phylogenic tree showed that the Iraqi isolate MW115895 clade was close to cluster of Hungary isolates (CP032160, CP032468) and USA isolates (CP054269) (figure 2).

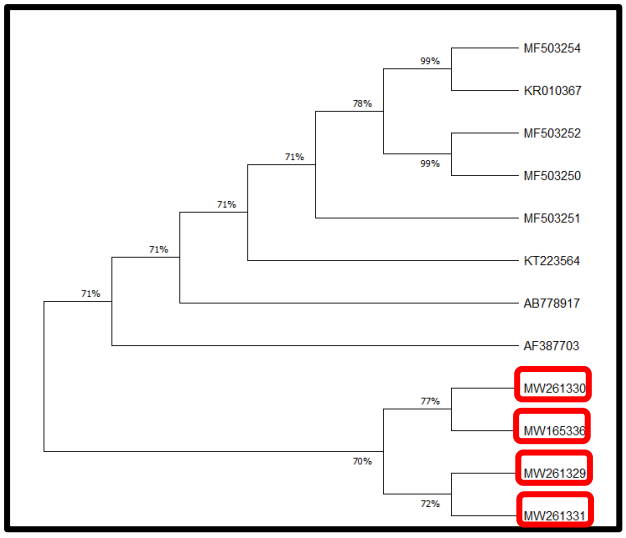

Figure 1: Phylogenic relationships among *Sarcoptis scabies assemblages* identified in infected internally displaced children lived in UNHCR camp. The analysis of the nucleotides sequences was conducted by a neighbor-joining method of 480 bp fragment of the ITS2 sequence. Genetic distances were calculated using the *P*- distance parameter model. Red rounded rectangle represents sequences generated in the present study. The obtained sequences aligned using Clustal W and phylogenic tree was conducted using MEGA10.

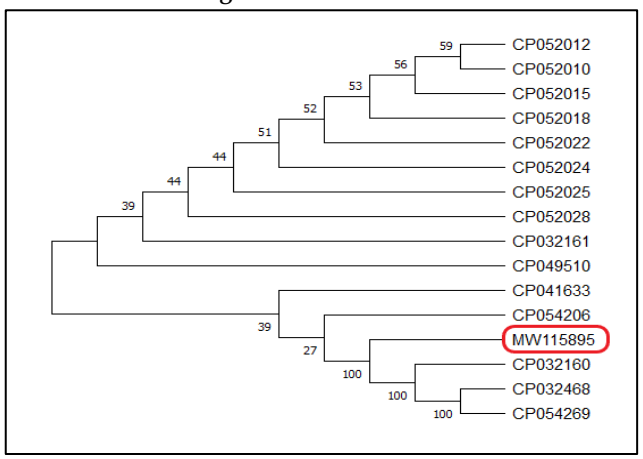

Figure 2: Phylogenic relationships among *Staphylococcus*

***aureus* identified in infected internally displaced children lived in UNHCR camp. The analysis of the nucleotides sequences was conducted by a neighbor-joining method of 310 bp fragment of the *mecA* gene. Genetic distances were calculated using the P- distance parameter model. Red rounded rectangle represent sequence generated in the present study. The obtained sequences aligned using Clustal W and phylogenic tree was conducted using MEGA10.**

4. Discussion

Various studies illustrated the prevalence of scabies among populations, refugee and internal displacement camps (Heudorf *et al*., 2016; Ozaras *et al*., 2016; Hussein *et al*., 2017). This study showed that the prevalence of scabies was 47.3% indicating a high frequency of scabies among internal displacement children visiting the health clinic at Cham Mishko camp. The rate recorded in the current study is higher than the rates obtained in previous studies performed in different parts of Iraq (Alaa, 2002; Mahmood, 2011). The tents build in Cham Mishko camp were emergency shelter designed with standard guide provided by UNHCR organization in which the shelter space/person is 3.5 m^2 and the minimum of ventilation areas is 1-1.5 m^2 between neighboring tents on all sides (UNHCR, 2000). The high percent of scabies in the current study in IDP might be due to prolong contacts between large number of people in each tent, in addition to poor hygiene and poor ventilation (Choli, 2017; Alberfkani and Mero, 2020). The rate of pyoderma associated with scabies was 39.4% as the most common secondary bacterial infection manifested as impetigo, folliculitis and furunculosis. In some studies, it was observed that people with scabies were 2.8 times more likely to develop impetigo (Esposito and Veraldi, 2018). Pyoderma were more common among families with large number of members, as they shar the same room, so they are more prone to develop scabies and impetigo (Romani *et al.,* 2017).

The close relationship between scabies and pyoderma was demonstrated in studies conducted among Australian Aboriginal community and the Solomon Islands in which the prevalence of scabies and pyoderma was reduced following treatment with permethrin cream (Carapetis *et al.,* 1999; Lawrence *et al.,* 2005). Many studies illustrated that the main causes of pyoderma in patients with scabies was *Staphylococcus aureus* followed by group A S*treptococcus* (Steer *et al*., 2009). Recently it was confirmed that scabies mites secrete complement inhibitors (SMSB4) that inhibit innate defenses, make their immediate surrounding favorable for growth of *S. aureus* and *S. pyogenes,* besides that, scratching the infested area with scabies mites lead to skin bacterial colonization and secondary bacterial infections (Swe *et al*., 2014; Swe *et al.,* 2017).

The most striking observation of the current study was that *S. scabiei* isolated from human were mainly distributed into two genetically distinct clades. The present study results agreed with other pervious analysis using ITS2 as DNA marker (Nas *et al*., 2018). These data suggested that mites belong to different clades are genetically isolated even if they were collected from humans living in the same area. The phylogenetic studies on *S. scabiei* from human using the ITS2 sequence are limited. Hence some sequences obtained from animal host were used in the tree analysis. The genetic differentiation between mites from human and mites from animals appeared substantial. Formerly, *Sarcoptes scabiei* is taxonomically divided into different varieties based on host origin (Fain, 1978). According to present study results and other research studies (Walton *et al*., 2004; Zhao *et al*., 2015) such varieties are no longer warranted. It is clear from analysis of sequences that humans were the initial source of domestic animals' scabies which in turn infested and become sources for other species of wildlife (Currier *et al.,* 2011). Diagnosis of *S. scabiei* in skin scrapings is relatively simple in case of crusted scabies patients due to presence of huge number of mites estimated to be from 1,000 to over a million. While there is a difficulty in the identification of mite's burrows in case of ordinary scabies since, there is

relatively few numbers of mites around 10 to 15 mites (Roberts *et al*., 2005). Therefore, microscopic examination of skin scraps has poor sensitivity as compared with PCR assay (Walter *et al*., 2011). Because it relies on visualization of scabies mites and their eggs within the skin scrapings, while PCR assay can detect excreta of mites and their cellular DNA within the stratum corneum cells of the host without the physical presence of mites in the skin scraping. Therefore, PCR assay has increased sensitivity over microscopic examination. In addition, the PCR assay could be potential during outbreak investigation of scabies when large number of suspected patients are screened.

Several molecular studies of *Sarcoptes scabiei* in humans and animals have targeted various genes such as microsatellites, ITS-2 ribosomal DNA (rDNA), mitochondrial 12S/16S rRNA, and *S. scabiei* myosin heavy chain genes (Fukuyama *et al*., 2010; Naz *et al*., 2013; Oleaga *et al*., 2013). PCR assay was positive in some patients who considered negative by microscopic examination. Hence, PCR technique can be considered as a recommended and confirmative method for diagnosis of scabies.

5. CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

6. References

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