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PRESENCE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN THE ENVIRONMENT AND WORKING PERSONAL AT DAIRY FARMS IN PESHAWAR (A CASE STUDY)

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ABSTRACT: *The MRSA is an opportunistic pathogen that has both medical and veterinary importance. Therefore, the present research was designed to investigate the prevalence of MRSA in farm environment and workers. A total number of 100 samples, 60 from Environment (floor n=20, feed n=20, water n=20) and 40 from workers (nasal n=20 and hand n=20) were collected from dairy farm in Peshawar. These samples were brought to Veterinary Research Institute Peshawar. The samples were cultured on the blood, nutrient and MacKoncy agar for the culture of S. aureus species. The positive cultures were subjected to biochemical tests for further confirmation. Total of 100 samples examined, 27 samples found positive and 73 samples were found negative for S. aureus. The prevalence of S. aureus isolates in environment sample was 13/60 (21.66%). Out of 13 positive samples, 11 (55%) from floor and 2 (10%) samples from feed were detected positive for S. aureus species. The collected water samples did not contain the organism. The samples obtained from personals exhibited prevalence 14 (35%) while the 26 (65%) sample were negative for S. aureus species. The presence of S. aureus in working personals hand samples found 30% and in nasal samples was recorded as 40%. MRSA was present in farm environment and working personal in randomly selected dairy farms in Peshawar district. The incidence of MRSA was recorded higher in farm workers in comparison to farm environment.*

Keywords: Farm, environment, workers, *Staphylococcus aureus*, mithicillin resistant,

INTRODUCTION

Staphylococcus aureus belongs to family Staphylococcaceae and is a common pathogen produce infections in humans and animals. The S. *aureus* is one of the most resistant pathogen among all non-spore forming pathogens. It has a strong ability to withstand to certain physical conditions such as salts concentration (7.5 – 10%), pH, temperature (60 ^{o}C for 60 minutes) air drying and some antibiotics [1]. The *S. aureus* is highly pathogenic and produces endo-toxins and exo-toxins e.g. exfoliative toxins, toxic shock syndrome toxin-1, *staphylococcal* enterotoxins, hemolysins (α, β, γ, δ) and leukocidins [2]. Exfoliative toxins act on skin and cause skin infections [3]. While enterotoxins usually associated with food poisoning [4].

In the early 1940s, the introduction of antimicrobial agent "penicillin" was efficiently used for the treatment of infections caused by *S. aureus* species followed by identification of penicillin-resistant species in 1942 [5]. In 1961 first semi-synthetic antibiotic Methicillin was used for the treatment of penicillin-resistant S. *aureus* but soon after (1961) a British scientist recognized first specie of *S. aureus* bacteria that was resistant to Methicillin called methicillin resistant *Staphylococcus aureus* (MRSA) [6].

Animal and human can acquire MRSA infection by contact with pus from an infected wound, skin and contaminated fomites. For many decades, MRSA was considered primarily a human pathogen, but the report of an MRSA infected dairy cattle in 1972 altered this perception [7]. Initial investigations described that the primary route of MRSA transmission between humans and animals. It was solely from humans to animals and majority of MRSA infections found in pets birds, cats, dogs, and horses were caused by human strains [8]. Once animal exposed to MRSA and may act as reservoirs and transmit to other animals and to their human handlers [9-10]. Main factors that play a vital role in the transmission of MRSA are crowding, skin scratches and abrasions, contaminated items or surfaces and poor hygiene [11]. The presence of MRSA in the environment might also be one of the sources of MRSA infection in animals, where it may survive for several months. Detection of MRSA from various environmental samples associated with animals including dust, farm rat, and environmental wipes [12]. The presence of *S. aureus* on the skin and mucosae of food-producing animals, such as ruminants, and the frequent association of the pathogen with mastitis, often leads to contamination of milk [13]. Contamination of milk can spread the organisms into environmental surrounding during handling and processing [14].

The acquired resistance (Methicillin resistance) to the β lactamase stable β-lactam antibiotics is caused by penicillin-binding protein PBP2a and PBP2b [15]. These are membrane-bound enzymes that target all β-lactam antibiotics. The PBP2a has low affinity to bind β-lactam antibiotics that enable *S. aureus* strains to acquire resistance against all β-lactams including methicillin, penicillin's and cephalosporin's [16]. PBP2a is encoded by a complex called mecA gene that contains insertion sites for plasmids and transposons and make possible to acquired cross-resistance to other non β-lactam antibiotics such as gentamicin, erythromycin, ciprofloxacin and clindamycin [17-18]. Furthermore, strains of MRSA have been identified that have property of multiple drugs resistance [19]. Some of the MRSA strains were found susceptible to non β-lactam antibiotics [20]. Keeping in view, this study was designed to investigate the prevalence of methicillin resistance

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staphylococcus aureus (MRSA) in farm environment and workers at randomly selected dairy farms in Peshawar, Khyber Pakhtunkhwa, Pakistan.

MATERIALS AND METHODS

A total number of 100 samples, 60 from environment and 40 from workers were collected from different cattle farm in Peshawar. Environmental samples included 20 floor swabs, 20 water and 20 feed samples were collected. Floor swab samples were and kept in liquid amies medium. Water sample were collected in a sterile McCartney bottle. Feed sample were collected in sterile polythene bag. Worker samples included 20 nasal swabs and 20 hand swabs samples were collected and stored in liquid amies medium. All the samples were kept in ice cooled container and were brought to the laboratory of Pathology and bacteriology Veterinary Research Institute (VRI) Peshawar.

The samples were cultured on various media such as nutrient agar (Oxoid), Mannitol salt agar (oxoid), Blood agar (Oxoid), Tryptic soy broth (Oxoid) and triple sugar iron agar (Oxoid). The growth of the organism was recorded on the different media and colonies were taken for the Gram staining characteristics. The different biochemical and sugar utilization tests were performed for the isolation and confirmation of the organism in different samples. The biochemical tests such as aesculin test, coagulase production, catalase production, gelatin liquefaction, methyl blue, indole production, bile tolerance, urease production, methyl red, triple sugar iron agar utilization, Hugh and Leifson's medium utilization, Vogous Proskauer, nitrate utilization, oxidase, Simmon's citrate, test, and sugar fermentation reaction were performed using procedure described by authors in [21-22]. The confirmation of MRSA isolates were performed using antibiotic sensitivity test according to instructions reported by CLSI (2007) [23]. Antibiogram of the isolates was carried out by kirby-Bauer disk diffusion method using cefoxitin (FOX 30ug) oxoid and oxacillin (OX 1ug) antibiotics. A suspension from the grown colonies was evenly distributed on Mueller Hinton agar plate and incubated at 37°C for 24 hour. Antibiotic discs were firmly dispensed over the surface of the agar, with an equal distance from each other. After 24 hours incubation Methicillin resistant *Staphylococcus aureus* were confirmed by formation of zone around oxacillin $\leq$ 14 mm and for cefoxitin $\leq$ 21 mm.

RESULTS AND DISCUSSION

Prevalence of *Staphylococcus aureus* isolated from environment at dairy cattle farm

In this investigation, a total number of 100 samples were collected from Environment and workers. Of the total samples, 60 sample were collected from environment i.e. floor, feed and water (20 samples from each) while remaining 40 sample were collected from nasal and hands of the workers (20 samples from each). Total of 100 samples examined, 27 samples were found positive with a prevalence of 27 % while 73 samples were found negative for *S. aureus*. The prevalence of *S. aureus* isolates in environment samples was 13/60 (21.66%). Out of 13 positive samples, 11 (55%) from floor and 2 (10%) samples from feed were detected positive for *S. aureus* species (Table- 1). Water samples did not contain the organism and found that water used at the dairy farms was free from the organism. The present study results are consentient to the results obtained by Jakee *et al.*, (2008) [24]. They investigated nasal swabs samples collected from persons working at different dairy farm and recorded 20% prevalence of *S. aureus*. Another study reported 20% of healthy human acts as carriers for *S. aureus* species, 30% acts as intermittent carriers while 50% were never infected with *S. aureus* species [25]. Similarly the present research work is in accordance with the results obtained by Mekuria *et al*., (2013) [26], they identified *S. aureus* species from nasal swabs of farm workers in different dairy farms. A total number of 68 nasal swabs were collected from farm workers, examined and recorded 13.2% prevalence of *S. aureus* positive isolates. However Sakwinska *et al.*, (2011) [27], reported 38% prevalence of *S. aureus* in farm workers.

Prevalence of *Staphylococcus aureus* isolated from worker at dairy cattle farm:

This table shows prevalence of *S. aureus* in farm worker. The samples obtained from personals exhibited prevalence 14 (35%) while the 26 (65%) sample were negative for *S. aureus* species. The prevalence of *S. aureus* in hand sample was 30% while in Nasal samples it was recorded 40% (Table-2). Roberson *et al*., (1998) [28] isolated *S. aureus* species from various samples collected from different sites of dairy farm.

Table 1. Prevalence of *Staphylococcus aureus* isolated from environment at dairy cattle farm:

Source	Source of sample	No of all samples	No of positive samples (%)	No of negative samples (%)
Environment	Floor	20	11 (55%)	9 (45%)
	Feed	20	2 (10%)	18 (90%)
	Water	20	0	20 (100%)
Total		60	13 (21.66%)	47 (78.33%)

Table 2. Prevalence of *Staphylococcus aureus* isolated from worker at dairy cattle farm

Source	Source of sample	No of all samples	No of positive samples (%)	No of negative samples (%)
Worker	Hand swab	20	6 (30%)	14 (70%)
	Nasal swab	20	8 (40%)	12 (60%)
Total		40	14 (35%)	26 (65%)

The prevalence of *S. aureus* recorded 70% from milk, 39% from dairy body sites, 28% from environmental sites and 16% from unidentified sources. There was presence of the organism has been reported in dairy farms managers, milking containers, water, feed and routine equipments used at dairy farm [29-31].

CONCLUSION

In summary, MRSA was prevalent in farm environment and working personal in randomly selected dairy farms in Peshawar district. The incidence of MRSA was recorded higher in farm workers in comparison to farm environment.

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