Community and Hospital Acquired Infection of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Erbil City

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ABSTRACT

Background and Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most frequently isolated pathogens in both community and hospitals, and associated with high morbidity and mortality rates with rapid development of resistance. The methicillin -resistance occurs due to the presence of PBP2a of the bacterial cell wall, which has low affinity for β-lactam antibiotics. MRSA are often multi-resistant to both β-lactams and non-β -lactams antibiotics. The study was documented the occurrence of community and hospital acquired MRSA infections.

Method: The clinical specimens were collected from patients at three teaching hospitals in Erbil city. All *Staphylococcus aureus* were identified as MRSA by detection of PBP2a.

Results: Out of 377 *Staphylococcus aureus* isolated, 30.24% were MRSA. The wound was the most common infection site for both community and hospital acquired MRSA. Statistically the patients with hospital acquired MRSA were older than the community acquired MRSA.

Conclusions: MRSA is one of the most common causes of serious infection in community and hospital settings. The most common site infected by MRSA is the surgical wound infection.

Keywords: MRSA, *Staphylococcus aureus*, community acquired, hospital acquired.

INTRODUCTION:

Staphylococcus aureus is well adapted to the human body, capable of spreading from person to person [1]. It is a serious human pathogen responsible for life-threatening septicaemia, endocarditis, and toxic shock syndrome [2]. The differentiation of MRSA strains from other strains of *Staphylococcus aureus* has important implications for the treatment and management of patients with *Staphylococcus aureus* infections [3].The methicillin-resistance occurs due to the presence of an additional penicillin-binding protein (PBP2a, also termed PBP2′) on the bacterial cell wall, which has low affinity for β-lactam antibiotics [4]. PBP2a, as a transpeptidase, facilitates cell wall concentrations of β-lactams inhibitory to native PBPs. MRSA strains cross-resistant to all β-lactams soon emerged in health care settings and made cephalosporins in addition to penicillins ineffective [5].

The most common infections caused by MRSA include surgical site infections, lower respiratory tract infections, urinary tract infections and skin infections [6]. Community acquired MRSA differs from the traditional hospital acquired MRSA in its antibiotic susceptibility profile [7].

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PATIENTS AND METHODS:

During the period March 2008 to March 2009, the clinical specimens including wound, urine, diabetic foot, skin abscess, and sputum were collected from 1189 patients at Hawler, Maternity and Rizgary teaching hospitals in Erbil city. The infections were classified into the community and hospital acquired MRSA.The wound swabs were collected from traumatic and surgical sites of all those patients that showed clinical evidence of wound infections. The specimens were collected using standard collection techniques [6]. Each patient was carefully instructed regarding the collection of a mid-stream urine sample. All patients with an indwelling urinary, the urine was collected through the draining portal of the urinary catheter using aseptic precautions with a sterile syringe. The samples were processed using cultural and direct examination techniques [8, 9]. Collections of diabetic foot specimens were performed in deep layers using cotton swab after skin washed with physiologic solution [10, 11]. Skin abscess cultures were obtained by swabbing the abscess cavity with a double-tipped culture swab [12]. The sputum specimens were collected from patients who showed signs and symptoms of lower respiratory tract infections. The specimens were processed using cultural and microscopically techniques [13]. Specimens were inoculated onto blood agar and mannitol salt agar. The plates were incubated aerobically at 37ºC for 18 to 24 hours.

Detection of *Staphylococcus aureus*: *Staphylococcus aureus* was identified [14] on the basis of:

-Blood agar (Oxoid, England): Cultures of *Staphylococcus aureus* typically yield golden-yellow colonies that are usually β-hemolytic on blood agar [15].

-Mannitol salt agar (Oxoid, England): Mannitol salt agar was used for screening device for *Staphylococcus aureus*, which is inhibitory to the growth of most bacteria A yellow zone surrounding their growth [16].

-Gram stain: *Staphylococcus aureus* appear as Gram-positive cocci, usually in irregular, often grape-like clusters [(15)].

-Tube coagulase test was performed by dispense 0.5 ml of human plasma in to a sterile tube by inoculation a loopful of the organism in to the tube then incubated at 35ºC in water bath for 4 hours, the tube were observed for clotting at intervals during the first 4 hours, over night incubation at room temperature were tested if no visible clot was observed after 4 hours. Known positive and negative controls were set in parallel [(17)].

-AVIPATH® STAPH agglutination test (Omega, UK) was used for detection of *Staphylococcus aureus* [(18)], which done as follows:

1. One drop of the isotonic saline was placed onto one of test circles.
2. The sterile loop was used to pick 2-4 colonies of the suspected *Staphylococcus* bacteria and emulsify in the isotonic saline on the test circle.
3. The latex reagent was vigorously shake, added one drop of reagent to the test circle then the reagent and culture emulsion by a disposable stirrer.
4. Gently and evenly, the test slide was rotated for 1 minute whilst examining the test slide for agglutination.
5. The slide was examined under a strong light source after 1 minute. A positive result was indicated by the obvious agglutination pattern of the latex in a clear solution. A negative result was indicated by no change in the latex suspension on the test slide.

Detection of MRSA: PBP2a kit (Oxoid, Japan) was used for detection PBP2a by using *Staphylococcus aureus* colonies from Mueller-Hinton agar (HiMedia, India), the presence of PBP2a is responsible for methicillin-resistance [(19, 20)] as follows:

A. PBP2a extraction

1. Two hundred µL of extraction reagent 1 were added into a tube.
2. A loopful of bacterial cells was suspended in the tube.

3. The tube was placed into boiling water for 3 minutes.
4. The tube was removed and allowed it to cool to room temperature.
5. One drop of extraction reagent 2 was added into the tube and mix well.
6. Centrifuge the tube at 1500xg for 5 minutes and used the supernatant for the test.

B. Latex agglutination

1. For each supernatant was tested, one circle of the test card were label for testing with test latex and another for testing with control latex.
2. The latex reagents were mixed well by inversion several times, and one drop of test latex or control latex to each labelled circle was added.
3. Fifty µL of the supernatant was placed on the test circle and the control circle. The latex and supernatant in each circle were mixed thoroughly with a mixing stick.
4. The card was picked up and rocked for up to 3 minutes and were observed for agglutination under normal lighting conditions.

C. Interpretation

1. PBP2a positive (MRSA): Agglutination was observed with test latex but not observed with the control latex within 3 minutes.
2. PBP2a negative (MSSA): No agglutination was seen with either the test latex or the control latex within 3 minutes.

RESULT:

Among 377 *Staphylococcus aureus* were isolated then identified, the proportion of MRSA infections among all *Staphylococcus aureus* isolates was 30.24% during the study period. Fifty-six isolates (24.24%) met the definition of community acquired MRSA infections and the rest 58 (39.73%) were classified as hospital acquired MRSA, statistical analysis (P<0.001) showed that the differences were significant between community and hospital acquired infections (Table 1).The total numbers of MRSA and common infection site for both community acquired (32.61% MRSA and 67.39% MSSA) and hospital acquired (40.21% MRSA and 59.79% MSSA) followed by urine (24.14% for community acquired MRSA and 38.46% for hospital acquired MRSA). Other MRSA infected sites included diabetic foot (23.88%), skin abscess (19.61%, which only isolated from the community), and sputum (21.05% of community acquired MRSA and 40% of hospital acquired MRSA). Statistically show that the differences were not significant (P= 0.071).

The specimens of 206 male (129 community acquired and 77 hospital acquired) and 171 females (102 community acquired and 69 hospital acquired) were identified as *Staphylococcus aureus*. The gender distribution among MRSA were 57.89% male and 42.11% female, while among MSSA were 53.23% male and 46.77% female. Males made up 62.5% of the community acquired MRSA and 53.45% of hospital acquired MRSA, whereas females made up 37.5% of the community acquired MRSA and 46.55% of hospital acquired MRSA, while the differences were not significant (Table 3). The range of patient ages was between 18 and 69 year, the age groups divided into 5 groups. A highest MRSA isolates (27.19%) were obtained from the age group of 60 $\geq$ years followed by age group 50 to 59 years (24.56%). MRSA infections were more often increasing with age, statistically the patients with hospital acquired MRSA were older (51.81±12.55 years) than the community acquired MRSA (46.14±14.52 years), statistical analysis (P=0.033) revealed that MRSA patients were older than MSSA patients (Table 4).

Table 1: Distribution of MRSA and MSSA isolated from community and hospital acquired infections

Staphylococcus aureus	**Community acquired**		**Hospital acquired**		**Community and hospital acquired**	
	n	**%**	***n***	**%**	***n***	**%**
MRSA	56	24.24	58	39.73	114	30.24
MSSA	175	75.76	88	60.27	263	69.76
Total	231		146		377	

High significant between community and hospital acquired (X^2= 10.17, P<0.001).

Table 2: Distribution of MRSA and MSSA isolated from clinical samples

***Staphylococcus aureus* infections**		**Clinical specimens**											
		Wound		**Urine**		**Diabetic foot**		**Skin abscess**		**Sputum**		**Total**	
		n	**%**	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**
Community acquired	**MRSA**	15	32.61	7	24.14	16	23.88	10	19.61	8	21.05	56	24.24
	MSSA	31	67.39	22	75.86	51	76.12	41	80.39	30	78.95	175	75.76
	Total	46		29		67		51		38		231	
Hospital acquired	**MRSA**	39	40.21	15	38.46	–	–	–	–	4	40.00	58	39.73
	MSSA	58	59.79	24	61.54	–	–	–	–	6	60.00	88	60.27
	Total	97		39		–		–		10		146	
Total	**MRSA**	54	37.76	22	32.35	16	23.88	10	19.61	12	25.00	114	30.24
	MSSA	89	62.24	46	67.65	51	76.12	41	80.39	36	75.00	263	69.76
	Total	143		68		67		51		48		377	

1. Not significant between community acquired MRSA and MSSA infections (X^2= 2.57, P= 0.0633).
2. Not significant between hospital acquired MRSA and MSSA infections (X^2= 0.04, P= 0.982).
3. Not significant between MRSA and MSSA infections (X^2= 8.62, P= 0.071).

– = not detected.

Table 3: Gender distribution among community and hospital acquired MRSA

Gender	Community acquired					Hospital acquired					Total				
	MRSA		MSSA		Total	MRSA		MSSA		Total	MRSA		MSSA		Total
	n	%	*n*	%		*n*	%	*n*	%		*n*	%	*n*	%	
Male	35	62.5	94	53.71	129	31	53.45	46	52.27	77	66	57.89	140	53.23	206
Female	21	37.5	81	46.29	102	27	46.55	42	47.73	69	48	42.11	123	46.77	171
Total	56		175		231	58		88		146	114		263		377

1. Not significant in the community acquired (X^2= 1.33, P= 0.249).
2. Not significant in the hospital acquired (X^2= 1.33, P= 0.249).
3. Not significant in the community and the hospital acquired (X^2= 0.70, P= 0.404).

Table 4: Age groups distribution among community and hospital acquired MRSA.

Age (years)	Community acquired					Hospital acquired					Total				
	MRSA		MSSA		Total	MRSA		MSSA		Total	MRSA		MSSA		Total
	n	%	*n*	%		*n*	%	*n*	%		*n*	%	*n*	%	
18-29	9	16.07	30	17.14	39	5	8.62	16	18.18	21	14	12.28	46	17.49	60
30-39	10	17.86	34	19.43	44	7	12.07	17	19.32	24	17	14.91	51	19.39	68
40-49	12	21.43	37	21.14	49	12	20.69	13	14.77	25	24	21.05	50	19.01	74
50-59	12	21.43	38	21.71	50	16	27.59	19	21.59	35	28	24.56	57	21.67	85
60 ≥	13	23.21	36	20.57	49	18	31.03	23	26.14	41	31	27.19	59	22.43	90
Total	56		175		231	58		88		146	114		263		377
Mean ages± SD	46.14±14.52[a]		44.73±14.20[a]			51.81±12.55[b]		47.31±14.52[ab]			49.03±13.79		45.59±14.59		

1. Significant between total MRSA and MSSA (T= 2.14, P= 0.033).
2. The same letters mean no significant difference.
3.The different letter mean significant difference at P<0.05.

DISCUSSION:

The prevalence of MRSA has increased worldwide, as it is evident from many surveillance studies. However, there are considerable differences between countries. The very highest rates of MRSA isolates have been noted in developed countries. While the prevalence of MRSA is lower in developing countries as in Africa [21].The present study revealed that out of 377 of *Staphylococcus aureus* isolates, 30.24% were MRSA for both community and hospital acquired infected. Comparable patterns have been seen worldwide as evident from the many recorded surveillance studies. MRSA is a well known cause of infections in Turkey and the overall prevalence of MRSA in seven centres was 33%, ranging from 12% to 75% [22]. Antibiotic Resistance Surveillance and Control in the Mediterranean Region reported that the overall median MRSA proportion was 39%. The highest proportions of MRSA were reported by Jordan, Egypt and Cyprus, where more than 50% of the invasive isolates were methicillin-resistant. Considerable variation was identified in the proportion of MRSA in hospitals within the same country [(23)]. In Riyadh, Saudi Arabia, found that the prevalence of MRSA in six major hospitals ranged from 12% to 49.4% [(24)]. MRSA had risen up to 43% in Nemazi Hospital, Shiraz, Iran [25]. However, the effectiveness of MRSA control depends on several factors, such as the existence and correct application of hygiene protocols to prevent transmission, level of care needed by patients, and antimicrobial drug prescription policies, which might differ between hospitals in a country [26].The frequency of MRSA isolates estimated in this study was significantly higher in the hospital than in community acquired infections. The trend of increased MRSA rates among hospital acquired isolates relative to community acquired isolates was observed in other studies [27, 28.] This study demonstrated that the infected males with to the study were reported that 60.7% males and 39.3% females [29]. Similarly, report 63% of MRSA isolation from males and 37% from females so this probably reflects the distribution of MRSA with a male patient predominance most likely due to the fact that exposure is greater [30]. In this study, the mean age of community acquired MRSA patients was 46.14±14.52 years that lower than hospital acquired MRSA patients (51.81±12.55 years). Similarly found that community acquired MRSA patients were younger than hospital acquired MRSA patients, the median age of community acquired MRSA patients was still significantly younger than hospital acquired MRSA patients [31, 32]. Investigated that the younger have a higher prevalence of community acquired MRSA as compared to older, who have a higher prevalence of nosocomial MRSA [33].Approximately 41.5% of the isolates came from patients in the extreme age groups, 21.0% were $\geq$ 60 years [24], which is compatible as in present study. The high prevalence in older patients might be associated with higher rates of chronic illness and hospital contact in older age groups [34]. On the other hand, other studies found not significant differences in the age distributions of individuals with MRSA infections were observed [35, 36].

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