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EFFICACY OF ANTI-BACTERIAL COMPOUNDS AND PLANT EXTRACTS AGAINST METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATES FROM NOSOCOMIAL INFECTIONS

M. Q. Zeshan, M. Ashraf, M. O. Omer, [*]H. M. Khan, [**]M. A. Ali and [**]A. A. Anjum

Department of Pharmacology and Toxicology, [**]Department of Microbiology, University of Veterinary and Animal Sciences, Lahore, 54000, Pakistan; [*]Department of Pharmacy, Lahore College for Women University, Jail Road, Lahore, 54000, Pakistan.
Corresponding Author's email: aftab.anjum@uvas.edu.pk

ABSTRACT

Sensitivity of Methicillin Resistant *Staphylococcus aureus* (MRSA) isolates purified from post operative wounds (n=100) from patients in surgical wards of Mayo, SIMS and Jinnah Hospital in Lahore-Pakistan was evaluated for antibiotics and plant extracts. The minimum inhibitory concentrations of ethanol extracts of medicinal plants (*Opuntia delinii, Acacia nilotica* and *Aloe vera*) and antibiotics (Moxifloxacillin, Cefipime, Imipenem/Cilastatin and Ampicillin+Cloxacillin) were determined by micro broth dilution method. *Staphylococcus aureus* isolates were identified on the basis of colonial morphology, microscopic morphology and biochemical characteristics. Resistance to Methicillin recorded was 72, 63 and 66 percent in isolates recovered from Mayo, SIMS and Jinnah hospital patients, respectively. Methanol extracts of *Aloe vera, Opuntia delinii* leaves, bark and leaves of *Acacia nilotica* were dried. Activity of *Aloe vera* against MRSA was highest with MIC value 32.248µg/mL followed by *Acacia nilotica* leaves (62.5µg/mL) and then of *Acacia nilotica* bark (84µg/mL). The extracts of *Opuntia dillenii* leaves had very low anti-MRSA activity having highest MIC value (1228µg/mL). The MIC of Moxifloxacillin and Imipenem, Cefipime and Ampicillin+Cloxacillin calculated against MRSA isolates was 2.68, 2.85, 57.81 and 11.32 µg/mL, respectively. Results of few selected anti-bacterial compounds were in close association with Linezolid (1.61 µg/mL) and Vancomycin (2.43 µg/mL).Among selected anti-bacterial agents efficacy of Moxifloxacillin, Imipenem, Ampicillin+Cloxacillin and Cefipime was comparable with standards. Both anti-bacterial compounds and extracts of studied plants may efficiently be used as alternate therapeutics against MRSA isolates.

Key words: Methicillin resistant *Staphylococcus aureus*, Plant extracts, Anti-bacterial, Minimum inhibitory concentration and Nosocomial infections

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a public health hazard since its inception and has spread across the world as an indicator agent of Nosocomial infections. Post surgical infections of patients in orthopedic units are mainly (48%) caused by *S. aureus* and 68% by MRSA (Eseonu *et al.,* 2011). Prolonged hospital stays, indiscriminate and irrational use of anti-bacterial compounds prior to visit physicians are main factors of MRSA emergence (Huttner *et al.* 2012; Kaleem *et al.,* 2010). Estimated frequency of MRSA is much higher (61%) in areas with thick human population in different cities of the Pakistan (Hafiz *et al.,* 2002).

S. aureus is normal inhabitant on skin and mucous membranes in human beings. In certain instances, bacteria enter through abraded skin or mucous membrane and can cause abscesses, pneumonia, meningitis, endocarditis and septicaemia (You *et al.,* 2011). It is established fact that MRSA isolates resist to numerous available beta-lactam antimicrobials. This resistance is mediated by the *mec*A gene which codes for a penicillin binding protein (PBP)2a with low affinity for beta-lactams (Aklilu*et al.,* 2013).

MRSA emerged in1970s and its number increased dramatically by 1990s throughout the world. In recent years a major change in epidemiology of MRSA has been observed, with the appearance of cases in the community affecting people having no epidemiological connection with hospitals. The strains isolated from such cases are called Community-acquired or Community-associated MRSA (CA-MRSA). Isolates from these cases have both phenotypic and genotypic differences with classically health-care associated MRSAs (Roberts 2013).

Alkaloids, tannins and flavonoids extracted from different medicinal plants exhibit antimicrobial potential (Fernandez *et al.,* 2012). The *Acacia nilotica, Aloe vera* and *Opuntia dillenii* have been tried successfully for therapy of gram positive bacterial infections including those caused by MRSAs (Abd-El-Nabi, 1992).

Infections caused by MRSA isolates are treated by Vancomycin (glycopeptide antibiotic) routinely. It has been reported that MRSA is resistant to most of the antibiotics with the exception of Vancomycin and

Linzolid (Kaleem *et al.,* 2011). Some reports on resistance of MRSA to Vancomycin have been documented (Mélard *et al.,* 2013). Cefepime, Moxifloxacin, Imipenem and combination of Ampicillin with Cloxacillin are being practiced to limit the post operative infections (Jacobsen *et al.,* 2011). In view of these facts present work was planned to evaluate the efficacy of selected antibacterial agents and medicinal plant extracts against MRSA isolated from indoor patients of selected tertiary care hospitals.

MATERIALS AND METHODS

In-vitro efficacy of selected anti-bacterial agents (Moxifloxacin, Imipenem, Cefepime and Ampicillin-Cloxacillin) and medicinal plant extracts (*Opuntia dillenii, Acacia nilotica* and *Aloe vera)* was determined against Methicillin Resistant *Staphylococcus aureus* (MRSA) isolates. Tested MRSAs were isolated from post-operative wounds of indoor patients.

Isolation of MRSA: Pus samples (n=100) were collected aseptically from surgical wards of Mayo hospital (n=40), SIMS (n=30) and Jinnah Hospital (n=30), Lahore-Pakistan from post-operative wounds of indoor patients admitted not less than five days. The samples were processed for identification of *S. aureus* using cultural, morphological and biochemical characteristics. Resistance of *S. aureus* isolates to methicillin was determined by disc diffusion method following the protocol described by Anupurba *et al.* (2003). Briefly, Staph.110 medium was used to check the resistance of isolates against methicillin. Bacterial suspension (01 mL) having $5x10^5$ colony forming units was spread over the surface of solidified agar under sterilized conditions. Methicillin (05µg) impregnated discs were placed over the inoculated culture plates and incubated for 24 hours. The isolates showing no zone of inhibition around methicillin discs were declared as MRSA.

Plant extracts: Leaves of three medicinal plants (*Opuntia dillenii, Acacia nilotica* and *Aloe vera*) were collected and got identified from Herbarium, Government College University, Lahore. Ethanol extracts of medicinal plants (leaves of *Opuntia dilenii, Acacia nilotica, Aloe vera* and bark of *Acacia nilotica*) were prepared using Soxhlet apparatus. All the extracts were filtered through membrane filter of 0.22µm pore size in a safety cabinet to avoid bacterial contamination **(**Wittschier *et al.,* 2009). Ethanol extracts were solidified by evaporating ethanol in a hot air oven.

Minimum Inhibitory Concentrations: Minimum inhibitory concentrations of plant extracts and antibiotics were determined by broth micro dilution method in 96 wells microtitre plates. Stock solutions (2%) of powdered plant extracts were prepared in normal saline (0.89%). Concentrations of medicinal plant extracts and antibiotics used were 4-2048µg/mL and 0.9-200 µg/mL. To prepare the inoculums, fresh colonies of MRSA were re-suspended in phosphate buffer saline. Optical density was adjusted to 0.5 McFarland standards and further diluted by 1:100 times in LB broth. Each dilution of antibiotic solutions (50µl) was added in 96 well micro-titration plate wells and inoculated with previously prepared MRSA inoculums (100µl). Positive controls contained only the media and inoculums without antibiotic, while negative control had media only. Plates were incubated aerobically at 37°C for 24 hours. Minimum inhibitory concentration values for each of the antibiotics and medicinal plant extracts were recorded (Modarresi-Chahardehi *et al.,* 2012).

Chi-Square test was used for the statistical analysis of the data.

RESULTS AND DISCUSSION

Staphylococcus aureus causes infections of skin, respiratory system, blood, soft tissues, intestinal and urinary tracts (Verhagen *et al.,* 2013).Therapy of bacterial infections fails due to acquisition of resistance against anti-bacterial agents (Martins *et al.,* 2013). Percent of septic wounds by methicillin resistant *S. aureus* (MRSA) infection was recorded 66% on the basis of microscopic studies, biochemical profile and resistance to methicillin. Infection of wounds by MRSA recorded was little higher (27/40=72%) in patients of Mayo hospital than Jinnah (20/30=66%) and SIMS (19/30=63%). However, the difference observed was statistically non significant (p=0.932). Difference among patients admitted in different surgical wards of the hospitals was non-significant (p=0.936). Butt *et al.* (2013) recorded 34.6% prevalence of MRSA infection in patients admitted in a tertiary care hospital Karachi. Hafiz *et al.* (2002) recorded different MRSA infection frequency in different parts of the country; 61%, 57%, 46%, 36%, 32%, 26% and 2% in Lahore, Karachi, Rawalpindi, Peshawar, Azad Kashmir, Quetta and Sukkur, respectively. Therapeutic use of antibiotics in post operative wounds was non-significantly associated with MRSA isolation (p= 0.652). Between genders, non-significant association of MSRA was observed (p= 0.461). Higher prevalence rate of MRSA infection in males as compared to females has been reported by Halablab *et al*. (2010); Tacconelli *et al*. (2004) and Selvey *et al.* (2000). Continuous use of methicillin resulted in the emergence of MRSA strains. Most strains *of S. aureus* are resistant to penicillin (Dezfulian *et al.,* 2012)**.** Forty two percent prevalence of MRSA was reported in Lahore-Pakistan by Hafiz *et al.* (2002). Percent prevalence of 70 and 37 was reported in different cities of India by Anupurba *et al.* (2003) and Rajaduraipandi *et al.* (2006).

Age of the patients was found insignificantly related to MSRA isolation (p= 0.427). Out of 66, bacterial isolates 10, 20, 18, 7, 5 and 6 were obtained from patients having age of 11-20, 21-30, 31-40, 41-50, 51-60 and 61-70 years, respectively. Statistically non-significant difference was observed in relation to span of stay in hospitals (p= 0.218). Same findings have been reported by Davis *et al*. (2014). In contrast McClelland *et al*. (1999) reported higher percentage (14.5) of MRSA infection among elderly people than young *i.e.* 6.3.

The patients suffering from appendicitis had highest infection with MRSA (14/14) followed by bullet fire wound (3/3), gynecological surgery (10/12), diabetic foot wound (9/11), bed sour (5/8), burn case (6/11), throat surgery (4/8), chest surgery (5/8) Colostomy (6/15), and hernia (4/10).Different parameters are presented at table (01).

Table 1: Prevalence of methicillin resistant *Staphylococcus aureus* in post operative hospitalized patients of tertiary care hospitals.

		MRSA			
		Positive	**Negative**	**Chi-Square**	**p-value**
N		66	34		
Gender	Male	39	19	0.095	0.461
	Female	27	15		
Age (years)	11-20	10	19	5.965	0.427
	21-30	20	7		
	31-40	18	9		
	41-50	7	3		
	51-60	5	4		
	61-70	6	1		
	71-80	0	1		
Hospital Stays (days)	1-5	10	12	8.291	0.218
	6-10	15	9		
	11-15	13	6		
	16-20	7	3		
	21-25	13	3		
	26-30	6	1		
	31-35	2	0		
Hospitals	Mayo	27	13	0.141	0.932
	Jinnah	19	11		
	SIMS	20	10		
Diseases	Colostomy	6	9	20.765	0.014
	Hernia	4	6		
	Bed Sour	5	3		
	Appendicitis	14	0		
	Burn case	6	5		
	Chest surgery	5	3		
	Diabetic foot wound	9	2		
	Gynecological surgery	10	2		
	Throat surgery	4	4		
	Bullet fire wound	3	0		
Wards	South surgical Mayo Hospital	6	4	4.906	0.936
	North surgical Mayo Hospital	8	2		
	East surgical Mayo Hospital	6	3		
	West surgical Mayo Hospital	7	4		
	Surgical ward 1 Jinnah Hospital	5	2		
	Surgical ward 2 Jinnah Hospital	5	2		
	Surgical ward 3 Jinnah Hospital	5	2		
	Surgical ward 4 Jinnah Hospital	4	5		
	Surgical unit 1 SIMS	4	3		
	Surgical unit 2 SIMS	4	3		
	Surgical unit 3 SIMS	6	1		

Antibiotics	Surgical unit 4 SIMS	6	3	6.858	0.652
	Ciprofloxacin	10	8		
	Augmentin	15	7		
	Imepenem	9	4		
	Linzolid	7	5		
	Ampicillin	3	2		
	Amoxicillin	3	3		
	Metronidazole	1	1		
	Vancomycin	12	1		
	Moxifloxcin	5	3		
	Levofloxacin	1	0		

Sensitivity of plant extracts against MRSA isolates was checked for four selected antibiotics (Moxifloxacillin, Cefipime, Imipenem and Ampicillin plus Cloxacillin) in comparison with Linezolid and Vancomycin. The minimum inhibitory concentration (MIC) of Moxifloxacillin and Imipenem, Cefipime and Ampicillin+Cloxacillin calculated against MRSA isolates was 2.68, 2.85, 57.81 and 11.32 µg/mL, respectively. The MICs of Moxifloxacillin and Imipenem were close to Linezolid (1.61 µg/mL) and Vancomycin (2.43 µg/mL). Anti-MRSA activity of Moxifloxacillin, Imipenem, Ampicillin+Cloxacillin and Cefipime was comparable with standards.

Vancomycin is extensively used in MRSA infected wounds (Allen and Nicas, 2003). Most of the selected MRSA isolates were sensitive to vancomycin (99%) and strengthened the observations of Kaleem *et al.* (2011). In contrast, intermediate level of resistance against the vancomycin was recorded by Mélard *et al.* (2013). Similar pattern of intermediate resistance of MRSA to vancomycin was analyzed by Vidaillac *et al.* (2013) and Assadullah *et al.* (2003) in India (6%). Calculated values of MIC (vancomycin) against MRSA ranged from 0.5 - 4µg/mL and drug may be the choice agent against MRSA.

Linezolid, a standard drug, showed 100 percent effectiveness against the MRSA as no isolate found to be resistant. In corroborations findings for efficacy of linezolid were recorded by Fatholahzadeh *et al.* (2008) in Iran and Kaleem *et al.* (2010) in Pakistan. In agreement results were declared by Hannan *et al.* (2009) for effectiveness of linezolid against MRSA. The MIC values of linezolid calculated ranged from 0.37 to 3µg/mL. Tunger *et al.* (2004) recorded 2 µg/ml MIC of linezolid against MRSA isolates. MRSA isolates resistant to vancomycin were susceptible to linezolid and may be a better choice for treatment of infections (Kohno *et al.*, 2007).

Minimum inhibitory concentration of moxifloxacin against MRSA was 2.68 µg/mL that fall within the range (MIC 0.25-4 µg/mL) reported by Farrell *et al.* (2011). Resistance of MRSA to the fluroquinolones has been observed by Biedenbach *et al.* (2010). Reported Moxifloxacin inhibitory concentrations (0.5 to 2µg/mL) by Morrow *et al.* (2011) at New Jersey were in accord with present work. Production of alternate target enzymes and drug efflux by the microorganisms were possible resistant mechanism (Bolon, 2009). Moxifloxacin was found to be effective as an alternative to vancomycin and linezolid against MRSA. Variations in the MIC values of imipenem against MRSA isolates were recorded. Observed lowest and highest values were 0.39 and 6.25µg/mL. Comparable results were reported by Fan *et al.* (1986) with activity range of 0.049-50 µg/ml. Few MRSA isolates showed resistance against imipenem so will not be a better choice for therapy. Cefepime MIC value (4µg/mL) was much higher than reported by Huang *et al.* (2004). Variation may be due to resistance developed by MRSA against this antibiotic. However, higher MIC (256 µg/mL) was observed by Queenan (2007) for cefipime and is in agreement with present findings. All isolates were sensitive to different concentrations of cefipime (25-100 µg/mL) as compared to standard drugs.

Combination of amoxicillin and cloxacillin exhibits better activity against the multi drug resistant bacteria (Cantoni *et al.*, 1989). Combination was checked against MRSA isolates and results were in line. However, activity was much lower. Antibacterial activity of *Acacia nilotica* and *Aloe vera* plant extracts against MRSA isolates was comparable with vancomycin and linezolid. It is suggested that extracts can be used as alternate therapeutic agents against MRSA.

In another experiment sixteen MRSA isolates were selected to assess the sensitivity pattern of bacteria against ethanol extracts of *Opuntia dillenii, Acacia nilotica* and *Aloe vera*. All tested isolates showed sensitivity to these extracts. The MICs of plant extracts against MRSA isolates were also calculated. Activity of *Aloe vera* against MRSA was highest (32.248µg/mL) followed by *Acacia nilotica* leaves (62.5µg/mL) and *Acacia nilotica* bark (84µg/mL). Extracts of *Opuntia dillenii* leaves had very low anti-MRSA activity with highest MIC value (1228µg/mL).

Active ingredients (terpenoids, saponins, alkaloids and glycosides) present in plant extracts have antibacterial activity against different bacterial strains. Most MRSA strains showed susceptibility to extracts of

Acacia nilotica leaves at MIC (62µg/mL). Concentration of leaves extract reported by Arias *et al.* (2004) was higher (125µg/mL). In accord MIC of *Acacia* leaves (39-78 µg/mL) was recorded by Khan *et al*. (2009). Average value of MIC for *Acacia nilotica* bark was found to be less than leaf extracts. It was observed that few strains showed the susceptibility at half concentration of bark extracts as compared to leaves. Results for *Acacia* bark extracts against MRSA are in agreement with findings of Eldeen *et al.* (2010). *Opuntia dillenii* exhibited low activity as compared to*Acacia nilotica* and *Aloe vera* against MRSA.

Aloe vera is used in cosmetics and nutraceuticals for skin infections, burns and cuts. It stimulates the cell growth and helps in repairing the damaged cells (Lu *et al.*, 2013). Ethanol extracts of *Aloe vera* showed highest antibacterial activity against MRSA isolates (MIC: 32.25µg/mL). Comparable results for *Aloe vera* were recorded by Pandey *et al*. (2010) (MIC: 1-20µg/ml) against MRSA.

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