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STAPHYLOCOCCUS AUREUS (*MRSA*) IN INTRA-ABDOMINAL INFECTIONS: RESISTANCE PATTERN AGAINST DIFFERENT ANTIBIOTICS

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ABSTRACT

Intra-abdominal infections (IAI's) a common cause of sever sepsis in the world and is caused by methicillin-resistant *Staphylococcus aureus (MRSA)*, which include pancreatitis, appendicitis, peritonitis and cholecystitis. The aim of current work was to study resistance of *MRSA* against various antibiotics. A total of 14 clinical isolates of IAI's patients were collected from public hospitals conformed using catalase and coagulase positive tests. Those isolates were evaluated for their resistance level against different antibacterial using agar dilution method. At concentration of 8 mg/l *MRSA* exhibited 100 % and 92.86 %sensitivity to linezolid and tobramycin while meropenem achieve this level at 128 mg/l ($p<0.05$) Tetracycline and cefuroxime give similar sensitivity levels against *MRSA* 128 mg/l and 8 mg/l .The mean sensitivity of *MRSA* at different concentrations of Linezolid was effective than levofloxacin while tobramycin also represent ~90 % mean sensitivity. MIC_{90} of linezolid and levofloxacin was $\geq 4mgl^{-1}$and$7mgl^{-1}$ respectively. Tobramycin and cefuroxime shows appropriate MIC›s of $\geq 6mg^{-}1$ and $5.5mgl^{-1}$ ($P<0.01$).The finding suggest that potency of antimicrobials against multidrug resistant *MRSA* of IAI's was linezolid> tobramycin >cefuroxime >levofloxacin>>…>Meropenem >tetracycline. IAI's can be effectively manage with above study drugs.

Key words: IAI's, Staph aureus (MRSA), linezolid, levofloxacin, tobramycin, cefuroxime, tetracycline, meropenem and Clinical Isolates (CI)

INTRODUCTION

Intra-abdominal infection(IAI's)are a multiple set of infectious diseases and is the 2nd most common cause of severe sepsis which leads to morbidity and mortality among (IAI's) patients. The complications related to IAI's includes appendicitis, diverticulitis, and peritonitis[1]. However Intra-abdominal infections involve appendicitis, diverticulitis, cholecystitis, gastroduodenal perforations, small bowl perforations, acute pancreatitis and peritonitis. Among these infections appendicitis is frequent and requires antimicrobial treatment and surgery[2]. Peritonitis is the inflammation of peritoneum, where bacteria, multiply in viscous fluid the infection caused by *Staph aureus* is very severe as compare to pathogens[3] cholecystitis (infection of gallbladder) caused by cystic duct obstruction and majority of peritonitis cases are caused by *Staph aureus (Methicillin resistant Staph aureus)*[1,4], Inflammatory condition of cholecystitis[5]. One of the common microorganism involve in causing above discussed IAI's is *Staph aureus* (*MRSA*)[2]), this pathogen is root cause of health care and community associated infections in the world which is a most challenging infection control issue [6]. The present work comprises of the study of alterations in antibacterial activity of linezolid, levofloxacin, tobramycin, cefuroxime, tetracycline and meropenem due to alterations in bacterial resistance and sensitivity.

Objectives of the present study: To observe the effect and potential of sensitivity pattern of some common drugs on the isolated pathogens of different intra-abdominal infections, and the increasing concentration of linezolid, levofloxacin, tobramycin, cefuroxime, tetracycline and meropenem on inhibition of colony forming unit (CFU) of *Staph*

aureus (*MRSA*) moreover to observe variations in MIC values of above drugs against *Staph aureus* (MRSA) of different IAI's.

MATERIAL AND METHOD

***Pathogens, media and antibiotics*:**14 isolates of *Staph aureus* were used in this study provided by microbiology laboratory, Jinnah Post Graduate Medical Center (JPMC) and Civil Hospital Karachi (CHK).

***Inclusion criteria*:** Age 12 to 65 years male and female**,** Isolates collection period from different IAI's patients 3.5 months.

***In-vitro studies*:** *Staph aureus* was grown and subculture on EMB agar and were further identified and conformed by catalase and coagulase positive tests. MIC values of linezolid, tetracycline, levofloxacin, tobramycin, cefuroxime, and meropenem were determined against 14 isolates of *Staph aureus*(*MRSA*) of various IAI's using the standers of national committee for clinical laboratory standers (NCCL) agar dilution method. The sub grown cultures were suspended in 1 ml Moller Hinton Broth, after 2 hours the broth acquired turbidity which showed bacterial growth and was matched with 0.5 MacFarland turbidity standards which contain 1×10^8 CFU/ml[7]. Series of concentrations i.e. 0.5, 1, 2, 4, 8, 16 $\mathrm{mg\,l^{-1}}$ for linezolid and levofloxacin 0.25, 0.5, 1, 2, 4, and 8 $\mathrm{mg\,l^{-1}}$ for tobramycin and cefuroxime and 2, 4, 16, 32, 64 and 128 $\mathrm{mgl^{-1}}$ for tetracycline and meropenem were prepared in double distilled water. All concentrations were mixed with liquid agar medium at (45 to 50^{o}C) in a ratio of 1:9 then it was poured in to sterilized petri plates near flame and were allowed for solidification[8] . Series of plates were prepared with addition of multiple inoculums by a replicator'sdevice [9]). After incubation (24 hours) MIC and number of resistant strains were observed and results were calculated by ignoring single colony or growth[10,11].

Statistical Analysis: Data was analyzed using SPSS program significant when confidence interval ($p\leq0.01$).

RESULTS

The results are compiled in table 1 and 2. *MRSA* at maximum concentration (16 mg/l) of Linezolid and levofloxacin exhibited0 % resistance, whereas similar % resistance i.e. 7.14 was observed at 8 mg/l and at 128 mg/l of tobramycin and Meropenem respectively Table 1. Tetracycline and cefuroxime at maximum concentration of also showed similar percent resistance i.e. 14.29 %. similar concentration of 8 $\mathrm{mgl^{-1}}$ *Staph aureus* (MRSA) give 100 % sensitivity to linezolid as compare to tobramycin which was 92.86 %. From figure 1 and table 1, While meropenem achieve this level at 128$\mathrm{mgl^{-1}}$, one way ANOVA and homogeneity of variances shows ($P<0.01$). In this study tetracycline and cefuroxime give similar sensitivity levels against *Staph aureus* (MRSA) but cefuroxime was superior in activity than tetracycline in case of concentrations which was 128$\mathrm{mgl^{-1}}$ and 8 $\mathrm{mgl^{-1}}$ for tetracycline and cefuroxime respectively. Cefuroxime may achieve 100 % sensitivity at concentration>8$\mathrm{mgl^{-1}}$. Figure 2, 3, and 4 shows the mean sensitivity at different concentrations which revealed that mean percent sensitivity was significant in case of linezolid and levofloxacin figure 4. Tobramycin also represent satisfactory percent mea sensitivity figure 2. MIC_{50} and MIC_{90} pattern oftested antibiotics against MRSA was linezolid> Tobramycin> Cefuroxime> Levofloxacin> Meropenem> Tetracycline, were comparatively lesser than levofloxacin as shown in table 1. Tobramycin and cefuroxime also represent MIC_{50} and MIC_{90} ($P<0.01$), while results of tetracycline and meropenem show that both antimicrobials achieve MIC at higher concentrations which is may be because of frequent use of these both antibiotics made *Staph aureus* (MRSA) more resistant as shown in table 1 homogeneity and one way ANOVA shows ($P<0.01$), mean for groups in homogenous subset display ($P>0.01$). **Table 2** shows the maximum concentrations at which *Staph aureus* (MRSA) isolates from different IAI's can be 100 % killed 100 % inhibition of *MRSA* isolates of different IAI's was calculated in Table 2. Linezolid at 8 mg/l shows 100% inhibition of *MRSA* against acute pancreatitis, appendicitis and peritonitis, while in cholecystitis it was achieved at 4 mg/l.

DISCUSSION

Emerging bacterial resistance is a worldwide concern[12]. Current antimicrobial studies revealed resistance trends from time to time which may help in the selection of empirical chemotherapy in the treatment of specific severe infections. Multi drug resistant *Staph aureus* (MRSA) CI from different IAI's were *n*=14 out of 80 isolates of three different pathogens, which constitute about 17.5 %. Fierobe in

his study of IAI's report MRSA isolates was about 21 % the difference was isolates were of postoperative IAI's [13]. In peritonitis our isolates of *MRSA* were 21.42 % while *MRSA* isolates in Western Australia in 12 month review were 22 % and in a 12 year spontaneous bacterial peritonitis was it was about 28 % [14,15]. Isolates of acute pancreatitis in our study were 35.71 %, while in another study of severe acute pancreatitis *Staph aureus* isolates were 17 % [16]. *MRSA* isolated from cholecystitis were 14.28 % in a case report *Staph aureus* was reported as a primary pathogen in acute pancreatitis[17]. We used concentrations between 0.5–16 mgl^{-1}. MIC_{50} and MIC_{90} of linezolid were achieved at 0.75 mgl^{-1} and ≥ 4 mgl^{-1} while[18] Livermore in 2003 find MIC's in-between 0.5 – 4 mg/l for *Staphylococci spp* and this was also retained for *MRSA*. Our study revealed that levofloxacin inhibit 90% (MRSA) at 7 mgl^{-1} as for as this MIC_{90} against *MRSA* was achieved at 16 mgl^{-1}, [19] . Tobramycin also show low MIC_{90} comparatively with levofloxacin which was ≥5.5mgl^{-1}, while 20 in 2005 Huang and Rybak[20] observed MIC at 128 mcg/ml ($P<0.01$). Cefuroxime inhibit 90 % microorganisms at 6mgl^{-1}. Cefuroxime MIC_{90} for *Staph aureus* (MRSA) was observed as 128 mcg/ml^{-1}, [21]. Meropenem and tetracycline achieve MIC_{90} at 80 mgl^{-1} and >96 mgl^{-1}. In a previous study meropenem achieve MIC_{90} at 32 mg/ml^{-1} [22] which was lower than our MIC_{90} which may be because of our pathogens were multidrug resistant form IAI's and also because of patients were already on broad spectrum antibiotics. Tetracycline MIC_{90} was greater in this study as compare with other chemotherapeutic agent of current study. But in other studies this MIC_{90} of tetracycline was very low like in 2010 Verhulst[23] reported MIC_{90} of tetracycline about 32 mgl^{-1}, which is very low as compare to our results. *MRSA is multidrug* resistant pathogens. *MRSA* of different IAI's represent variations in concentrations at which thy produce 100 % microbial growth inhibition. Acute pancreatitis, appendicitis and peritonitis shows similar concentration of 8 mgl^{-1},while in cholecystitis at 4 mgl^{-1} 100 % bacterial inhibition was attain as shown in Table 2. Baldoni *et al* also reported linezolid as active against *MRSA*[24]. Levofloxacin also represent comparatively good activity against *Staph aureus* (*MRSA*) of different IAI's as it has been suggested as an option of monotherapy in IAI's, which MIC was achieved at 1 mgl^{-1} [25,26], In present study MIC_{50} was achieved at 1 mgl^{-1} ($P<0.01$). Tobramycin inhibit 100 % *Staph aureus* (*MRSA*) growth at 8 mgl^{-1} and 4 mgl^{-1} ($p<0.01$) the reason for this difference was because of isolates were from different patients with different intra-abdominal infections. Digranes in 2009 report MIC ≤ 0.5 mgl^{-1} for *Staph aureus* but in this study pathogens were *Staph aureus* (*MRSA*) from IAI's patients and patients were already on chemotherapy[26]. Cefuroxime shows variations against *Staph aureus* (*MRSA*) of different IAI's. Endermann *et al* in 2007 also reported 2 mgl^{-1} the MIC of cefuroxime against *Staph aureus*[27] which was comparatively significant than our results. In current study the MIC_{90} of cefuroxime was 6 mgl^{-1} the reason for comparatively this higher concentration was because pathogens were multi drug resistant *Staph aureus* (MRSA) and patients were already on broad spectrum chemotherapeutic agents while Endermann use cefuroxime against methicillin susceptible *Staph aureus*. Linezolid and levofloxacin produce significant percent mean sensitivity ($P \leq 0.01$) as shown in Figure 4. Tobramycin and cefuroxime also give suitable percent mean sensitivity ($P \leq 0.01$) Figure 2. The trend among graph 2 and graph 3 show that with increasing drug concentration a linear sensitivity against *Staph aureus* (*MRSA*) was achieved. At 16 mgl^{-1} of linezolid and levofloxacin there was no resistance reported in current study against *MRSA* respectively. In table 2 there are marked variations in concentrations at which 100 % pathogens were killed of different IAI's interestingly majority of antibiotics shows low MIC's against *Staph aureus* (*MRSA*) of cholecystitis, which may be because of handling error or small sample size.

CONCLUSION

Intra-abdominal infections and their causative pathogens, its resistance is a global health problem; particularly multi dug resistant pathogen like *Staph aureus* (*MRSA*) are responsible for morbidity and mortality rate. Linezolid, levofloxacin, tobramycin, and cefuroxime are considered as potent drugs in management of IAI's. Data generated from this study could be valuable for the selection of therapeutic alternatives in health care problems.

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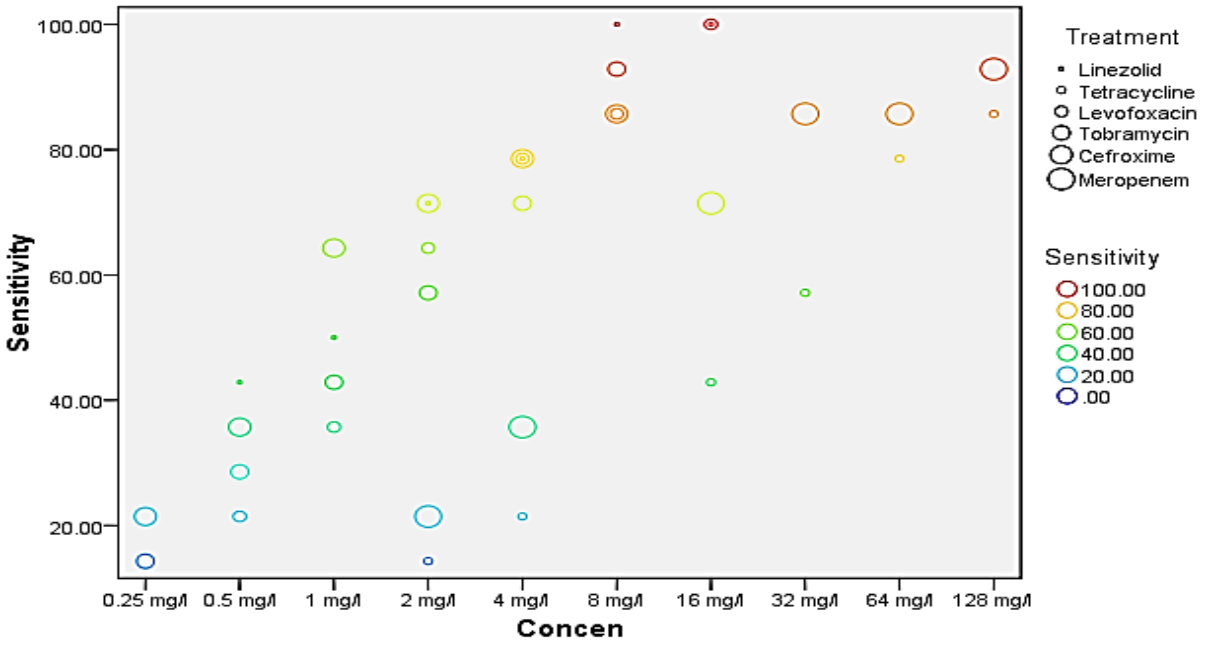
Table 1: Cumulative susceptibility of 14 multi-drug resistant *Staph aureus* (MRSA) strains of IAI's to Linezolid, Levofloxacin, Tetracycline, Tobramycin, Cefuroxime and Meropenem

Microorganism (*n*=14)	Antibiotics	Conc. Range $mg\,l^{-1}$	MIC_{50} $mg\,l^{-1}$	MIC_{90} $mg\,l^{-1}$	% Resistance
Staph aureus(MRSA)	Linezolid	0.5-16	0.75	≥4	0
	Tetracycline	2-128	16	> 96	14.29
	Levofloxacin	0.5-16	1	7	0
	Tobramycin	0.25-8	1	≥5.5	7.14
	Cefuroxime	0.25-8	0.5	6	14.29
	Meropenem	2-128	≤6	80	7.14

Table 2: Concentrations at which 100% inhibition observed.

Antimicrobials	Concentrations range $mg\,l^{-1}$	Conc. For 100 % Bacterial Killing of (*Staph aureus MRSA*) in Different IAI's $mg\,l^{-1}$
Acute pancreatitis (*n*=5)		
Linezolid	0.5- 16	8
Tetracycline	2-128	>128
Levofloxacin	0.5-16	16
Tobramycin	0.25-8	> 8
Cefuroxime	0.25-8	> 8
Meropenem	2-128	128
Appendicitis (*n*=4)		
Linezolid	0.5- 16	8
Tetracycline	2-128	>128
Levofloxacin	0.5-16	16
Tobramycin	0.25-8	8
Cefuroxime	0.25-8	> 8
Meropenem	2-128	96
Peritonitis (*n*=3)		
Linezolid	0.5- 16	8
Tetracycline	2-128	64
Levofloxacin	0.5-16	8
Tobramycin	0.25-8	8
Cefuroxime	0.25-8	4
Meropenem	2-128	64
Cholecystitis (*n*=2)		
Linezolid	0.5- 16	4
Tetracycline	2-128	64
Levofloxacin	0.5-16	4
Tobramycin	0.25-8	4
Cefuroxime	0.25-8	1
Meropenem	2-128	32



Concen= concentrations

Figure 1: Percent Sensitivity of different Antibiotics against *Staph aureus MRSA* at different concentrations.

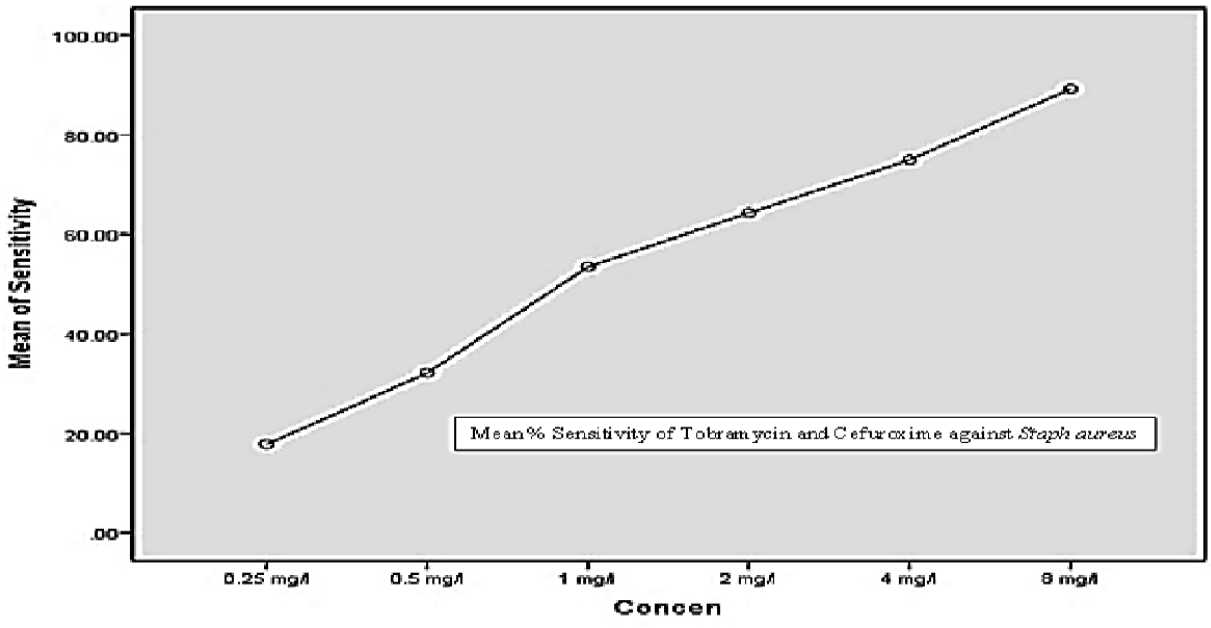


Figure 2: Mean percent sensitivity of tobramycin and cefuroxime against *Staph aureusMRSA*

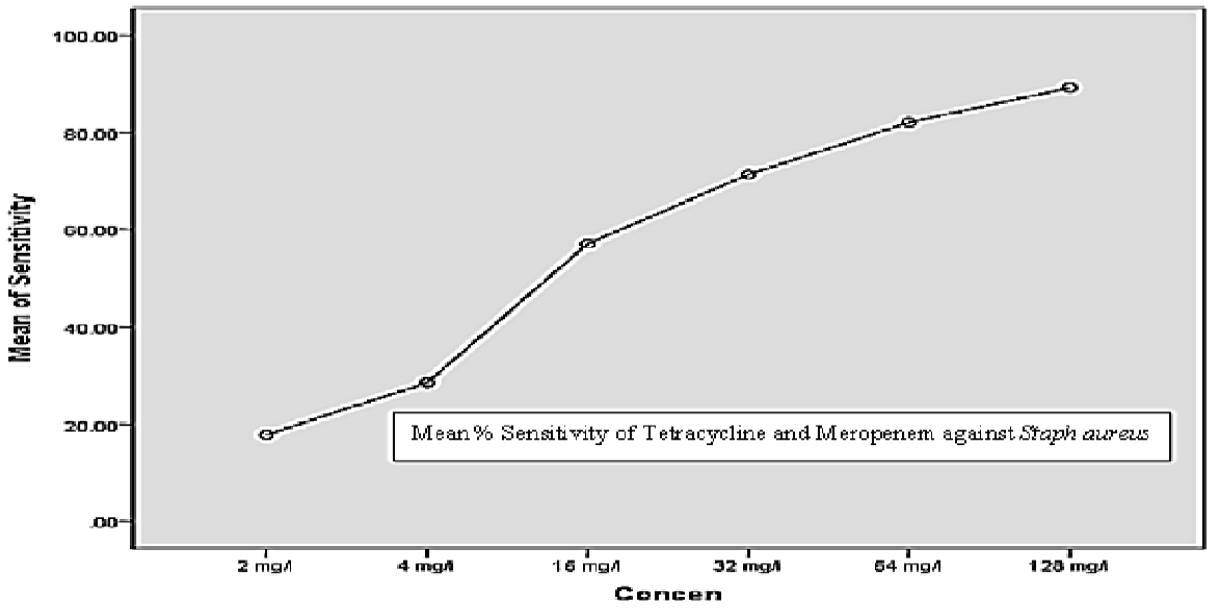


Figure 3: Mean percent sensitivity of tetracycline and meropenem against *Staph aureusMRSA*.

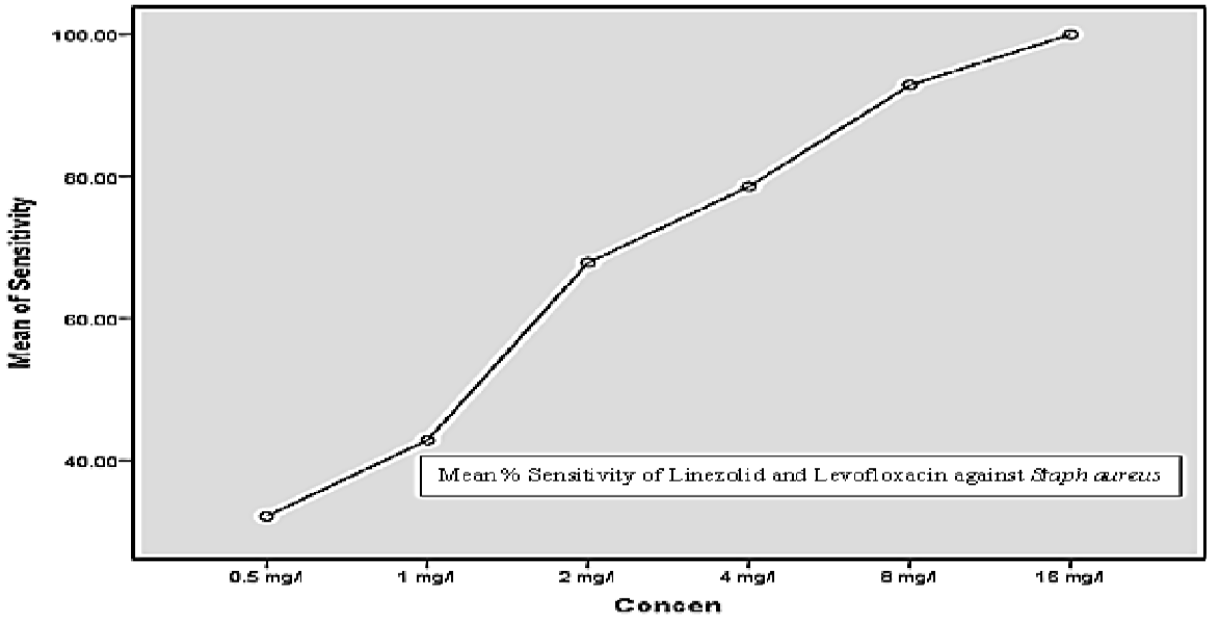


Figure 4: Mean percent sensitivity of linezolid and levofloxacin against *Staph aureus MRSA*.

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