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Vancomycin MIC variability among the *Staphylococcus aureus* isolates from the different sample types in Punjab

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Abstract
Background: *Staphylococcus aureus* isolates from a different sample type, including meat (chicken, chevon, and pork), swabs from butcher shops (Chopping knife, butcher hands, and chopping block), nasal swabs (bovine and swine), and swabs from community settings (Cell phone, Office telephone, Door handle, computer mouse and keyboard, chair arm and tap faucet), were observed for variability in vancomycin minimum inhibitory concentrations (MICs). High vancomycin MIC in Methicillin-Resistant *S. aureus* (MRSA) has been theoretically linked to treatment failure and may be a precursor to Heterogeneous Vancomycin-Intermediate *S. aureus* (hVISA) and Vancomycin-Intermediate *S. aureus* (VISA). This study aimed to determine existed variability of vancomycin MIC among *S. aureus* isolates, including MRSA, in Punjab.
Methods: MIC of vancomycin by E-test of all *S. aureus* isolates including MRSA isolates studied between Feb. 2014 to Aug. 2016.
Results: Three of the 201 examined isolates had MICs greater than 2 µg/mL, and all three came from community-based settings. Vancomycin was effective against the majority of MRSA isolates, with a MIC of 1 to 1.5 µg/mL. The MIC variability among the meat and swab samples did not differ significantly. However, the difference was considerable when the MIC variability of the swab from the community settings was compared to the meat and nasal/butcher shop swab samples.
Conclusions: The majority of the MRSA isolates were still highly susceptible to vancomycin, but when the sample type was examined, there was evidence of a considerable difference in vancomycin MICs.

Keywords: *S. aureus*, vancomycin MIC, MIC variability, MRSA, hVISA, VISA

1. Introduction
S. aureus is a normal microbiota component and does not infect individuals with good immune systems (Rasigade and Vandenesch 2014) [16]. However, MRSA, a challenging public health concern, can result from *S. aureus* invading internal tissues or the bloodstream. This is mostly because *S. aureus* can introduce and disseminate antibiotic-resistant bacteria (Taylor and Unakal 2017) [20]. MRSA infections are more likely to result in increased morbidity, a longer course of antibiotic therapy, an extended hospital stay, and a higher fatality rate as compared to infections brought on by Methicillin-susceptible *S. aureus* (MSSA) (Charles *et al.* 2004) [5].
The cornerstone of treatment for severe MRSA infections is vancomycin, the most often given antibiotic in the glycopeptide class of medicines (Soriano *et al.* 2008) [19]. Treatment failure with vancomycin is, however, being reported more frequently, even with isolates in the susceptible range. According to published research, vancomycin's MIC directly influences how MRSA infections progress. Patients with MRSA bacteremia isolates that have a higher MIC to vancomycin are more likely to have extended bacteremia and higher mortality (Soriano *et al.* 2008) [19].
Based on their lesser susceptibility to vancomycin, the Clinical and Laboratory Standards Institute (CLSI) separates the *S. aureus* isolates into three groups. These three strains of bacteria include vancomycin-susceptible *S. aureus* (VSSA), vancomycin-intermediate-resistant *S. aureus* (VISA), and vancomycin-resistant *S. aureus* (VRSA). To assess whether an isolate belongs to VRSA, molecular methods should be utilized to show the presence of *van*A or other *van* resistance determinants (Werner *et al.* 2008) [23].
Following the emergence of vancomycin-resistant *enterococci* in the 1980s, worries over the potential for large-scale epidemics of vancomycin-resistant *S. aureus* (VRSA) caused by the acquisition of the *van*A gene from *enterococc*i emerged (Rehm and Tice 2010) [17].

This concern was later confirmed by the effective transmission of the *van* gene from an *Enterococcus faecalis* to an MRSA strain in mice exposed to a variety of bacteria (Noble *et al.* 1992) [13]. In 2002, the USA saw the discovery of the first VRSA strain (Chang et al. 2003) [4]. Since then, 52 VRSA strains carrying *van* genes have been discovered, with 14 strains found in the United States (Askari *et al.* 2013) [1], 16 in India (Tiwari and Sen 2006, Thati *et al.* 2011) [22, 21], 11 in Iran (Azimian *et al.* 2012) [3], 9 in Pakistan (Azhar *et al.* 2017) [2], 1 in Brazil (Panesso *et al.* 2015) [14], and 1 in Portugal (Melo-Cristino *et al.* 2013) [12].

The *S. aureus* strain hVISA, which is described as an *S. aureus* strain with a vancomycin MIC within the susceptible range (≤ 2 µg/ml) while a cell subpopulation is in the vancomycin-intermediate range (≥ 4 g/ml), is thought to have originated VISA strains (Howden *et al.* 2010) [7]. It is well-accepted that VISA is caused by a steady accumulation of gene mutations (Katayama *et al.* 2016) [9]. Particularly important are the genes for two-component regulatory systems, such as *WalKR* (Peng *et al.* 2017) [15], *GraSR* (Yoo *et al.* 2013) [25], and *VraSR* (Hu *et al.* 2016) [8]. Even while various VISA strains have different genetic alterations, it is still unclear why they frequently display identical behaviors (McGuinness *et al.* 2017) [11].

According to Shariati *et al*`s meta-analysis study, the frequency of VRSA, VISA, and hVISA infections increased by 2.0, 3.6, and 1.3 times, respectively, after 2010. (Shariati *et al.* 2020) [18] and the graphic below illustrates the global predominance of these strains (Figure 1). This increase in the prevalence of VRSA, VISA, and hVISA can be attributed to a number of factors, including the environment and animal-derived foods.

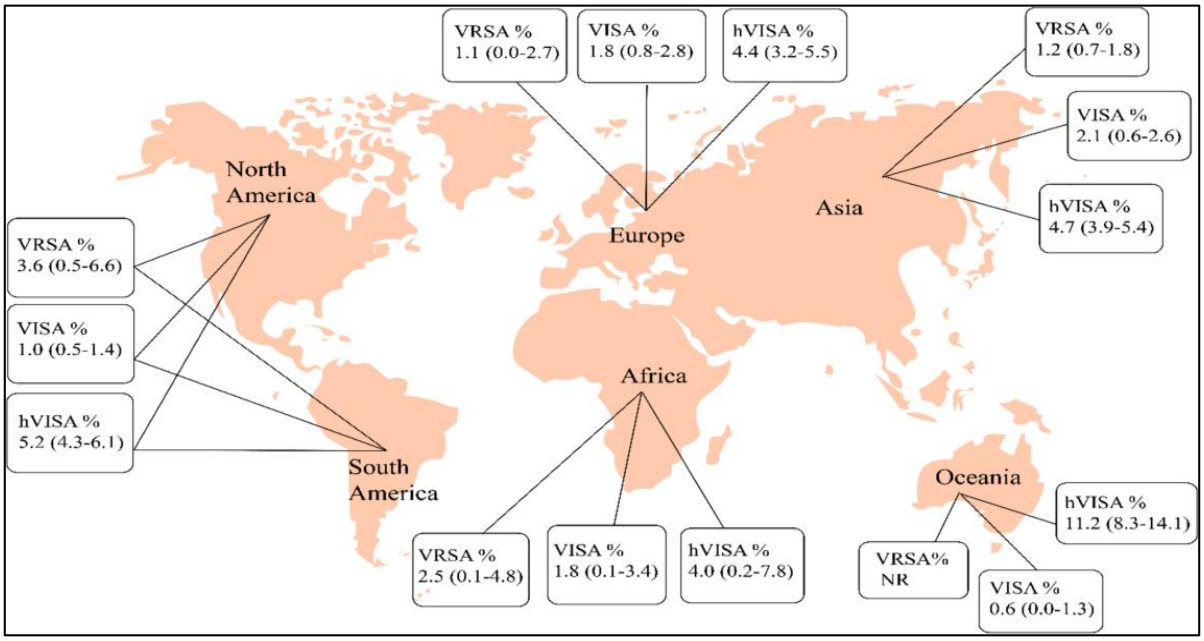

Fig 1: From "Global prevalence and distribution of VRSA, VISA and hVISA clinical isolates: a systematic review and meta-analysis" (Shariati *et al.* 2020) [18].

In Punjab, the behaviour of MSSA/MRSA isolates in relation to vancomycin MIC over sample type has not been studied. The current study's objective is to assess the vancomycin MIC distribution for isolates of MSSA/MRSA according to the type of sample.

2. Materials and Methods

2.1. Study samples

The *S. aureus* isolates from meat (chicken, chevon and pork) (Zehra *et al.* 2020b) [29], nasal swab samples (bovine/porcine) (Zehra *et al.* 2017) [28], swab samples from butcher shops (butcher hand, chopping block, and chopping knife) (Zehra *et al.* 2020b) [29] and swabs from community settings (Cell phone, Office telephone, Door handle, computer mouse and keyboard, chair arm, and tap faucet) (Zehra 2020) [26] were collected and analyzed from Feb 2014 to Aug 2016 in Center of One Health Laboratory, GADVASU.

2.2. Detection of methicillin resistance

S. aureus isolates were identified according to the CLSI guidelines and as per the study conducted by Zehra *et al* 2017, 2020a, 2020b [28, 27, 29].

2.3. MIC testing methods

For all isolates, the MIC of the vancomycin was calculated using the E-test method following the manufacturer's instructions. All analyses used the actual E-test MIC values. All of the MIC values were trended over the sample type.

2.4. Statistical analysis

For data entry and analysis, the Statistical Package for Statistical Sciences (SPSS) version 24.0 was utilized. Parametric ANOVA was used to evaluate continuous variables. If $p \leq .05$, a test was deemed significant. The graphic representation of the vancomycin MIC versus each type of sample was created using Microsoft Excel 2007.

3. Result

All the 201 isolates, irrespective of the sample type, were susceptible to vancomycin using the current CLSI guidelines except the three isolates from the community setting that showed MIC > 2 µg/mL (Zehra 2020, Zehra *et al.* 2020a) [26, 27]. The demographic characteristics of the isolates are presented in Table 1.

Table 1: Frequency distribution of the isolates against each vancomycin MIC value

VAN MIC	Isolates	MDR	MRSA	Chicken	Chevon	Pork	Total-meat	Nasal-Swab	Swab-butchers	Swab-Community
0.25	2	1	0	0	0	0	0	2	0	0
0.385	4	2	0	0	0	0	0	1	3	0
0.5	12	7	1	1	0	3	4	0	2	6
0.75	27	9	2	6	0	5	11	5	5	6
1	96	39	5	21	14	12	47	21	21	7
1.5	51	24	4	10	7	7	24	8	7	12
2	6	4	1	1	2	0	3	0	0	3
3	2	1	0	0	0	0	0	0	0	2
4	1	1	1	0	0	0	0	0	0	1
Total	201	88	14	39	23	27	89	37	38	37

Most of the MRSA isolates were from settings in communities (Zehra *et al.* 2020a, Table 1) [27]. The proportion of MRSA isolates with MIC values between 0.5 and 4 µg/mL varied, with 4 µg/mL being the highest value, and this was also true of MDR isolates (MIC 0.25 to 4 µg/mL). The following charts show this MIC variability of isolates in relation to sample type (Figure 2, 3).

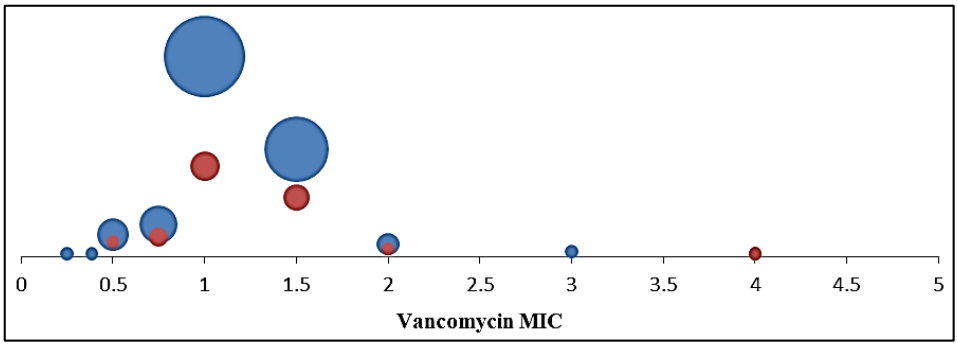

Fig 2: Distribution of MDR and MRSA, disaggregated by frequency of *S. aureus* isolates and the vancomycin MIC. Blue bubble size represents the number of MDR *S. aureus* among the total isolates and brown bubble size represents the number of MRSA among the total MDR isolates.

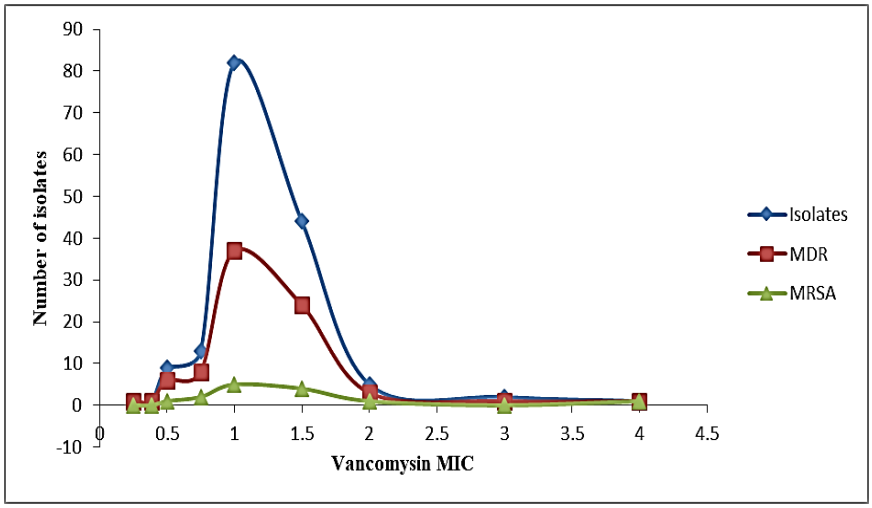

Fig 3: Distribution of *S. aureus* isolates, MDR, and MRSA, disaggregated by frequency of *S. aureus* isolates and the vancomycin MIC.

There were no appreciable differences in the MIC variability between the meat and swab samples. The difference was significant ($p<.05$) when the MIC variability of the swab from the community settings was contrasted with the meat and nasal/butcher shop swab samples. The following figures show the variation in vancomycin MIC (Figure 4, 5).

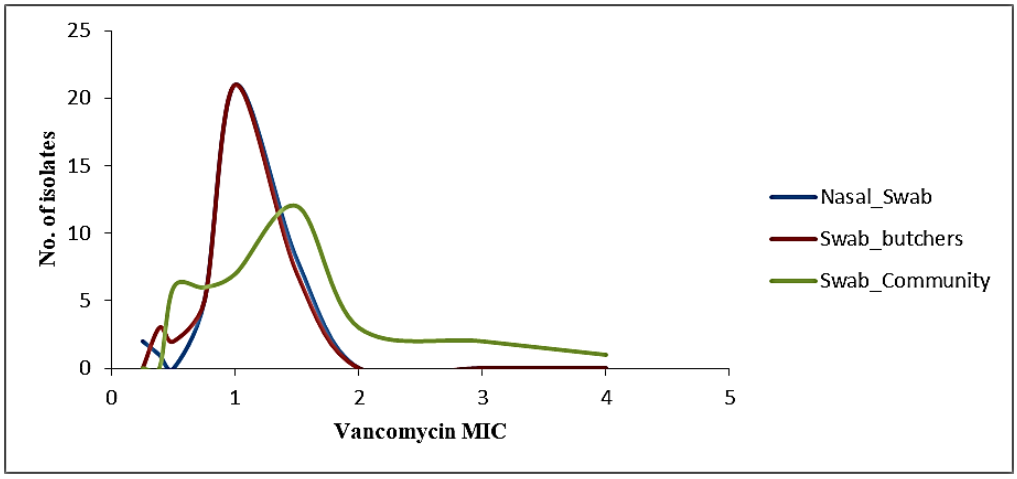

Fig 4: Distribution of *S. aureus* isolates corresponding to the swab sample type, disaggregated by frequency of *S. aureus* isolates and the vancomycin MIC.

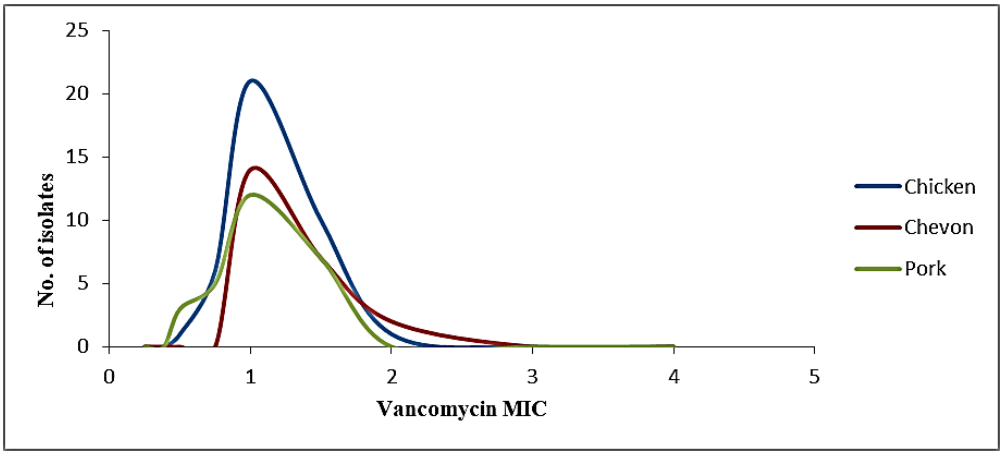

Fig 5: Distribution of *S. aureus* isolates corresponding to the meat sample type, disaggregated by frequency of *S. aureus* isolates and the vancomycin MIC.

4. Discussion

Antibiotic-resistant bacteria can be transferred from animals to humans through contact or the food chain. Additionally, through human gut flora, animal microbes can transfer resistance genes to human pathogens (McGuinness *et al.* 2017) [11]. When fed an avoparcin-containing diet, broiler chickens, developing pigs, calves, and beef cattle have all benefited from the growth-promoting properties of the drug. Avoparcin has also been used to treat necrotic enteritis in poultry (Hilde *et al.* 2009) [6]. It was shown that in countries where avoparcin was used for the aforementioned purposes, commensal microbiota of food animals and the meat from these animals usually contained vancomycin-resistant *enterococci (*VRE) (Hilde *et al.* 2009, Zehra *et al.* 2017, Zehra *et al.* 2020a, 2020b) [6, 28, 27, 29]. Vancomycin is used relatively seldom in hospitals however, VRE has been found in the commensal microbiota of healthy people (Wijesekara *et al.* 2017) [24]. Therefore, it is possible to envision the transfer of such resistant genes to *S. aureus.*

The current investigation demonstrates how *S. aureus* isolates from various sample types/sources varied in their MIC values for vancomycin. Figures show that the isolates from community settings have higher vancomycin MICs (Table 1, Figure 2). VISA is a community-based MRSA isolate having a vancomycin MIC of 4 µg/mL. Given the volume of studies demonstrating the shortcomings of vancomycin-based MRSA infection treatment, this is worrying (Tiwari and Sen 2006) [22].

Additionally, hVISA, defined as a *S. aureus* isolates with a vancomycin MIC in the susceptible range (≤ 2 µg/ml), while a cell subpopulation is in the vancomycin-intermediate range (≥ 4 µg/ml), is an isolate that is believed to have originated VISA isolates based on conventional methods (Howden *et al.* 2010) [7]. The present study's limitation is that, although 98.51% (198/201) of the isolates had vancomycin MIC ranges of 0.25-2 µg/ml, the vancomycin population analysis profile (PAP) could not be done.

In addition, depending on the isolate, there is a spectrum from VSSA to VISA in terms of the relative percentage of the cell population that is resistant to vancomycin at 4 µg/ml. For the exact detection of this feature, a PAP is required (Howden *et al.* 2010) [7]. When identifying the hVISA phenotype in *S. aureus* isolates with vancomycin MICs as low as 0.5 to 1 µg/ml, PAP can be utilized as a reference approach (Leonard *et al.* 2009) [10].

In summary, the current study is one of the few to demonstrate the variance in vancomycin MIC with respect to the source/type of sample, and it is clear that samples from community settings have a wider range of MIC than those taken from animals. However, before drawing any strong conclusions about the origins or kinds of samples that contribute to the transmission of the hVISA, VISA, and VRSA, a study with a larger sample size is required.

5. Conflict of interest
The authors declare no conflict of interest

6. Author's contribution
Zehra A.- Lab work, Data analysis, and Manuscript writing; Singh R.- A technical program of work; G K. M - Data visualization, Manuscript writing.

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