

Linezolid-resistance *Staphylococcus aureus* – Prevalence, Emerging Resistance Mechanisms, Challenges and Perspectives

Hassan Rafique¹, Nazim Hussain¹, Muhammad Usama Saeed¹,
Hafiz M.N. Iqbal^{2*} , Gulfam Azim³ and Muhammad Bilal^{4*} 

¹Centre for Applied Molecular Biology (CAMB), University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan.

²Tecnologico de Monterrey, School of Engineering and Sciences, Monterrey, 64849, Mexico.

³National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan.

⁴School of Life Science and Food Engineering, Huaiyin Institute of Technology, Huaian 223003, China.

Abstract

Staphylococcus aureus, an opportunistic pathogen, can root several infections viz skin and tissue infections, bacteraemia, food poisoning, pneumonia, and many other clinical conditions with some variations of virulence factors. In treatment of infections, caused by this Gram-positive pathogen, several antibiotics are being used importantly Methicillin and Vancomycin. This pathogen has high capability of antibiotic resistance development and had evolved new strains such as Methicillin-resistant *Staphylococcus aureus* (MRSA), and Vancomycin-resistant *Staphylococcus aureus* (VRSA). Meta-analysis in Ethiopia showed that pooled prevalence of MRSA in environment, food, animal, and human was 54%, 77%, 15%, and 38% respectively (2022). Risk of MRSA isolates from burn ICU was 55 % higher (2018). In Bangladesh 37.1% isolates from frozen meat chicken (2021) were identified as MRSA. This problem is being dealt with a novel drug called Linezolid which has been proved effective against both MRSA and VRSA. Exacerbating the situation, this pathogen has shown resistance against this unprecedented drug by means of a number of drug resistance mechanisms. Its prevalence has been reporting since the adoption of the drug, but with a minute ratio at one time/place to the very high percentage at another time/place. This inconsistent prevalence must not be ignored, and its surveillance should be augmented as antibiotic treatment is critical for fighting against microbial infections. This review highlights the worldwide reports in which *Staphylococcus aureus* of either wildtype or Methicillin or Vancomycin resistance that have shown resistance to Linezolid drug for the past 2 decades. At the same time where incidences of Linezolid Resistant *Staphylococcus aureus* (LRSA) indications are reporting, there is a call for comprehensive strategies to overcome this challenge of antibiotic resistance.

Keywords: Linezolid, Antibacterial Resistance, Linezolid Resistant *Staphylococcus aureus*

*Correspondence: hafiz.iqbal@tec.mx; bilaluaf@hotmail.com

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INTRODUCTION

Being a part of normal microbiota on skin, nasal cavity, and woman's LRT (lower reproductive tract), *Staphylococcus aureus* (*S. aureus*) is an unscrupulous pathogen whose colonization can be the origin of infections ranging from normal to critical and even lethal including endocarditis and bacteraemia.^{1,2} Accounting several clinical situations such as mastitis, sepsis, food poisoning, osteomyelitis, pneumonia, wound infections, abscess formation, toxic shock syndrome (TSS), meningitis, and also respiratory infections such as sinusitis, can be instigated by colonies formation of *S. aureus* bacteria.^{1,3} Disease index can be chiefly divided into wide-ranging infections such as: infections of the tissues and skin (cellulitis, impetigo & boils), infections of the lung and urinary tract, infection of deep sites (any organ e.g., spleen, heart valve, liver, bones, and joints etc.) and toxin-facilitated diseases (scaled skin syndrome, toxic shock syndrome, food poisoning). Toxin-facilitated diseases in this list range from self-restrictive to life-threatening. The complication arises when a condition known as bacteraemia is reached which refers to the presence of viable bacteria in blood and this can lead to even more dangerous condition known as sepsis. Further manifestations of nosocomial infection due to *S. aureus* include ventilator associated pneumonia, wound infection, bacteraemia associated with Intravenous devices, and other sorts of prosthesis associated infections (such as prosthetic joints, cerebrospinal fluid shunts and vascular graft).⁴ Being a non-spore forming opportunistic pathogen, with the resistance against environmental conditions, it is proficient to survive for indeed a long period even in a dry state. It can grow on temperature range between 7°C to 47.8°C. The optimal growth temperature for it is 35°C. The pH range for growth is between 4.4 & 9.3 while the optimum growth pH is 7.0 – 7.5. Moreover, it has extremely tolerant strains to sugars and salts.⁵ According to epidemiological studies, the basic habitat of this micro-organism is human anterior nares and about 30% individuals carry it there at any given time, but in case of patients and hospital personnel the rate may be higher.⁶ Another research showed that *S. aureus* bacterium (chiefly found in the nose) is temporarily

or persistently carried out by 30-50% population which is indeed a very large number.⁷

S. aureus can cause pathogenesis; therefore, it is quite important regarding various clinical aspects. The active antibiotics against *S. aureus* are Cephalosporins and Penicillin.⁶ The bacteria can generate resistance against harmful conditions either by getting the gene horizontally or with the help of mutations. The bacteria which do not have the resistance to a specific antibiotic can acquire gene or plasmid of resistant bacteria to that specific antibiotic. Naturally, *S. aureus* had evolved and started to produce an enzyme called beta lactamase (β -lactamase) which hinders the activity of Penicillin.⁸ These strains were called PRSA (Penicillin Resistance *Staphylococcus aureus*). Therefore, 1st generation Cephalosporins and new group of antibiotics i.e., β -lactam antibiotics comprising Penicillin G, Methicillin, Oxacillin, Nafcillin which suited well for such susceptible strains, became the choice of treatment.⁶ The pathogen somehow acquired resistance gene for such kind of antibiotics and a new strain called MRSA was reported. A study, conducted in the region of Pakistan (Karachi) reported 44.9% prevalence of MRSA.⁹ For the patients with β -lactam sensitivity, Vancomycin became the choice for the treatment of MRSA. Exacerbating the situation, there have been reports of the resistance of Vancomycin which emerged a new kind of *S. aureus* called VRSA.¹⁰ Emergence of such decreased Vancomycin susceptibility in strains is a major concern with respect to MRSA treatment with Vancomycin.⁶ Research is continuously reporting the prevalence of VRSA among patients, a formidable threat to the treatment of MRSA infections.² The study in Iran reported 11 VRSA strains out of 80 *S. aureus* strains and 1 VISA (Vancomycin Intermediate *Staphylococcus aureus*) which were obtained from clinical samples.¹¹ Hand in Hand, there had been the use of Linezolid drug for the treatment of these VRSA strains which is the elementary concern of us. This article is written in perspective of Linezolid drug use in treating infections of *S. aureus*, the mechanism associated with its Linezolid resistance, the key theme which is the prevalence of Linezolid Resistance *Staphylococcus aureus* (LRSA) strain in different places of the world, and the finally, the solution of ongoing challenges of Linezolid resistance.

Linezolid and *Staphylococcus aureus*

Because of the resistance generated by *S. aureus* against many groups of antibiotics, a novel drug called Linezolid (a representative drug of antibiotic group- oxazolidinone) is in use. Structural, physiochemical, and pharmacokinetic data of Linezolid are shown in Figure 1. It has the inhibitory activity against MRSA, VRSA, and GISA (Glycopeptide-Intermediate *Staphylococcus aureus*). This drug has been proved as effective cure against infections (Bacteraemia) caused by MRSA (Figure 2).¹² Clinical trials demonstrated that this unprecedented drug is an active cure for patients of community acquired pneumonia and nosocomial skin infections. Linezolid had showed itself as active as other well-established treatments. For example, oral dosage of Linezolid 400 mg twice daily is equal to Clarithromycin 250 mg twice daily in effectiveness for community acquired pneumonia or simple SSTIs.^{13,14} Linezolid is being used for the treatment of complex skin and tissue infections. This drug is considered

immensely suitable for the treatment of MRSA based complicated skin & soft tissue infections.¹⁵ Studies have shown a relatively effective activity of Linezolid against MRSA and strains which are resistant to glycol-peptides and other agents of antibiotics.¹⁶ It is reported that Linezolid is also effective against infections of MRSA strains which form biofilms (BF) on biomaterials. The efficacy of Linezolid against MRSA has been found competent in vivo with baicalein- a flavonoid which is used in Traditional Chinese Medicine.¹⁷

Linezolid has proved well effective against infections of *S. aureus* and many reports have been published witnessing its usefulness. Several infections of *S. aureus* can be treated with Linezolid. A study showed that Linezolid is as effective as Vancomycin in the treatment of children infections caused by Gram-positive bacteria.¹⁹ Another randomized study which compared Vancomycin and Linezolid for the treatment of bacteraemia caused by *S. aureus* was conducted. The pooled data showed that

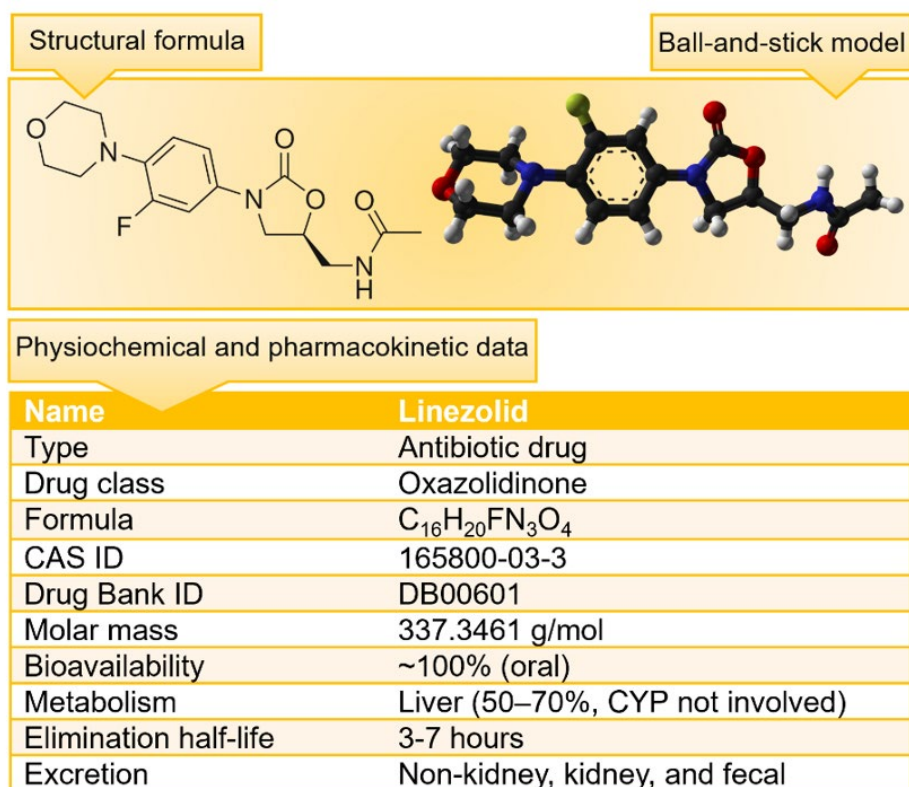


Figure 1. Structural, physiochemical, and pharmacokinetic data of Linezolid

the results of Linezolid treatment were not lesser than that of Vancomycin treatment.²⁰ Infections of Central Nervous System caused by MRSA may also be treated with Linezolid.²¹

In Japan, another report witnesses the safety and efficiency of Linezolid for the treatment of MRSA infections. The report also suggested that it is rather more efficient as compared to Vancomycin as an antibacterial agent for the eradication of micro-organisms.²² Fu et al.²³ analysed the measured trials for *S. aureus* through meta-analysis. His study of pooled data proposed that Linezolid was more efficient for the treatment of patients with SSTIs (Skin and Soft Tissue Infections) as compared to glycopeptides. A latest study was conducted to find out the safety and efficiency of Linezolid for the treatment of meningitis. The studied claimed that Linezolid appeared safe to them for the treatment of meningitis.²⁴

Mechanism of Linezolid Action

Even though the precise mechanism of Linezolid (oxazolidinones) has not been accomplished completely, but they are known to act through inhibition means in the bacterial protein synthesis' initial phase. The proposed concept behind the action is that the agents of this group of antibiotics interrupt fMet-transfer RNA interaction with the 50S subunit of ribosome at the time of preinitiation complex formation at very early stage.¹³ Thus, preventing the formation of 70S initiation complex and bacteria cannot synthesis proteins for survival and growth.²⁵ The main function of Linezolid is to inhibit the protein synthesis in bacteria, as the proteins are crucial for cellular metabolism and cellular structure,²⁶ the result is the inhibition of bacterial growth and multiplication.

However, due to the incomplete knowledge of linezolid selectivity through known structures of Linezolid complex with ribosomes of

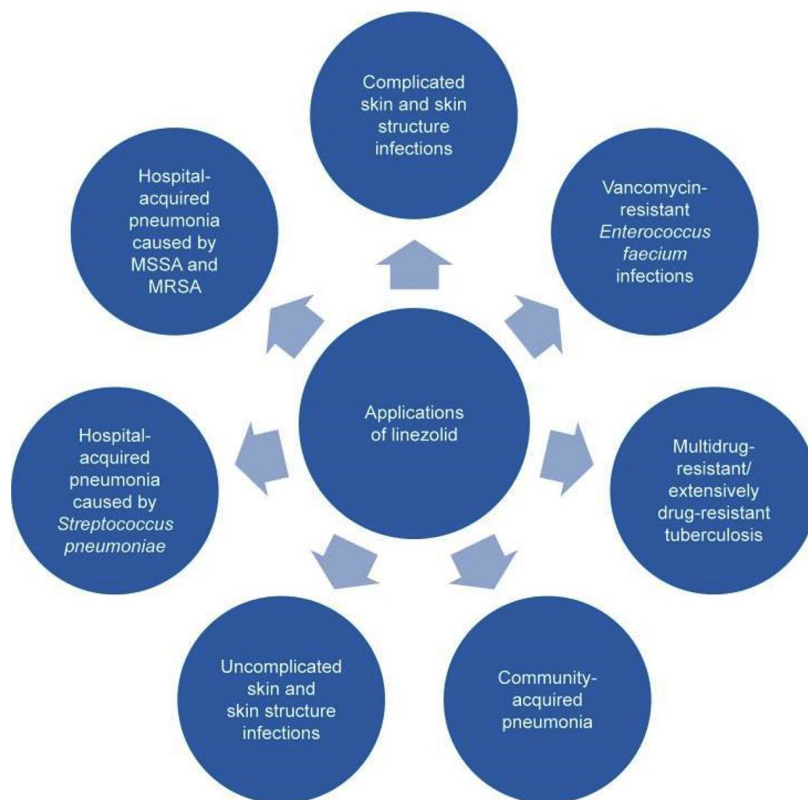


Figure 2. Applications of the linezolid. Reprinted from Ref.¹⁸ with permission under the Creative Commons Attribution - Non Commercial (unported, v3.0) License

bacteria led to the further studies through use of various technologies such as molecular dynamics simulation methods, for understanding of action of Linezolid. One of such study published the simulated structure complex of Linezolid with ribosome of *E. coli*. Their study contributed to the understanding of mechanism of translation inhibition of Linezolid.¹⁴

The site of action of oxazolidinone in bacteria is cytoplasmic ribosome while the molecular modelling study showed that oxazolidinones were non-interactive towards human cytoplasmic ribosomes, but obstruct mitochondrial protein synthesis, therefore, were expected to interact with the mitochondrial ribosomes. This drawback results in clinical side effects of oxazolidinones therapy.²⁷ For instance, respiratory enzyme content can be faded, if mitochondrial protein synthesis is inhibited, consequently, limiting the aerobic energy production. The outcome of the situation is an increase in anaerobic glycolysis and lactate generation despite of the absence of tissue hypoxia. This untoward interaction of antimicrobial drug and human mitochondrial ribosome becomes the root cause of Linezolid-induced lactic acidosis- that is rare but lethal side effect of Linezolid.²⁸

It is reported that with Linezolid, reduction in toxin production by Gram-positive pathogen is possible as Linezolid may also inhibit the expression of virulence elements. The interaction of Linezolid with other antimicrobials is minimal and thus it can be used simultaneously with other antimicrobials as well without the decrease in its efficiency.¹⁸

Effects of Linezolid Treatment

Linezolid is reported to have good penetration ability into cerebrospinal fluid and thus has a good therapeutic effect in health care associated meningitis.²⁹ On the other hand, serotonin toxicity can result with Linezolid if used with serotonin reuptake inhibitors as Linezolid is a nonspecific inhibitor of an enzyme called monoamine oxidase. Therefore, clinicians must be aware of this situation. Other clinically adverse effects associated with Linezolid may be anaemia (due to direct effect of Linezolid on red cell population of bone marrow), hyperlactatemia, thrombocytopenia, ocular and peripheral

neuropathy, hypoglycaemia, reticulocytopenia, diarrhoea and nausea.¹⁸

It is reported that Linezolid -at achievable concentration in serum- modulates the *in vitro* production of cytokines (IL-1 β , IL-6, IL-1ra and TNF- α), thus, having the immunomodulatory effect on human peripheral blood mononuclear cells. This effect along with other antimicrobial effect is protective in preventing the local destructive inflammatory response in areas of infection as well as in alleviating the symptoms of non-infectious inflammatory conditions.³⁰ Other preclinical evidence illustrated that Linezolid suppresses the secretion and phagocytic ability of immune cells as well as the expression of immune related genes at the mRNA level under the stimulation of pathogens or endotoxins.³¹

Bacterial Response to Linezolid Exposure

A report found dose-dependent reactive oxidation species (ROS) in MRSA strains after Linezolid exposure and demonstrated that the strains in study were not under oxidative stress environment due to Linezolid exposure. The result showed that the protein synthesis inhibition provokes deceleration of cellular respiration -crucial to metabolic activities- and the oxidative stress of each strain was associated with metabolic activity.³²

Development of Linezolid Resistance

Linezolid, a novel drug, was synthesized to treat bacterial infections prompted by Gram-positive bacteria. It is well suited as Vancomycin against MRSA. However, there are reports where *S. aureus* has developed resistance to such a novel drug and emerged a highly threatened strain i.e., LRSA. The emergence of resistance was considered a result of mutation (discrete nucleotide substitution) in V domain of 23S ribosomal RNA gene especially G2576T, T2500A and G2447T.³³ There is another scenario of Linezolid resistance which is non mutational and is due to acquired gene from plasmid of resistant strains of *Enterococcus faecalis*.

One such incident of transferable resistance in micro-organisms is discussed here to elaborate the acquired resistance in *Staphylococcus aureus*, and the accountable gene of for this scenario is recognized as *cfr* (chloramphenicol-

florfenicol resistance) gene. Linezolid is synthetic in nature, and it was supposed that no resistance gene pool would be existed in micro-organisms to hinder the clinical significance of the drug; though, the cause of its resistance in strains was considered a mutation but there are now reports which explain the transferable resistance against Linezolid.³⁴ The mechanism of drug action is that; it binds to bacterial ribosome at 50S subunit through interaction with 23S ribosomal RNA, thus obstructing the protein synthesis.³³ *Cfr* gene is considered responsible for multi-drug resistance. The product of the gene is an enzyme called methyltransferase which causes methylation of 23S rRNA gene region known as A2503 (large ribosomal subunit's gene). Thereby, promoting resistance to strains against drugs like florfenicol, chloramphenicol and clindamycin. This kind of resistance of *S. aureus* against drugs is called *cfr*-mediated resistance. The case of Linezolid resistance among *S. aureus* due to *cfr* gene was first reported by Toh et al.³⁴ The first strain of *S. aureus* to be resistant to Linezolid is known as CM05 named after its isolation place and date

(C=Colombia, M=Medellin, 05=2005). PCR-specific primers were used to identify *cfr* presence in the genome of pathogen's strain. Toh et al.³⁴ analysed antibiotic sensitivity of MRSA strains which is positive for *cfr* gene rather than the mutation in drug target site. The earliest report of *cfr* gene mediated resistance to Linezolid in *S. aureus* in clinical human isolates which were recovered in United States was published in 2008. This study was conducted by Farrell et al.³⁵ They claimed to describe first detection of *cfr* mediated resistance in human isolates during LEADER program (according to which about all strains (99.9%) of *S. aureus* isolated in US were Linezolid susceptible). In this study, bacterial strain was obtained from 45-year-old woman which was showing the symptoms of sepsis, pneumonia, and urinary tract infection. The woman was admitted to a hospital in Ohio and strain (004737X) retrieved from bronchio-alveolar lavage specimen, further the patient subsequently been given Linezolid, Vancomycin, Erythromycin & Piperacillin-tazobactam and kept in hospital for 1 month. According to the study isolate shown Linezolid resistant phenotype. This outcome led

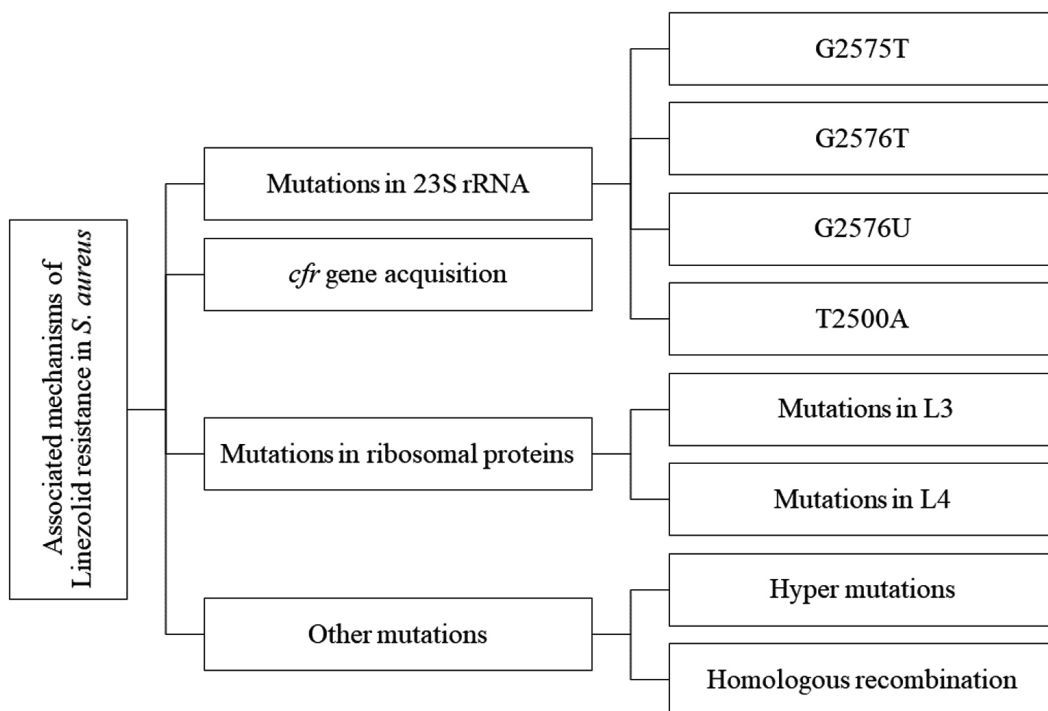


Figure 3. Associated mechanisms of Linezolid resistance in *Staphylococcus aureus*

to screening of G2576T mutation but that was not present. But the result was positive for *cfr* primers which was further confirmed by sequencing.³⁵

In 2010, a study reported first outbreak of *cfr* mediated Linezolid resistance in *S. aureus* conducted by Morales et al.³⁶ They experimentally explored the means of resistance in MRSA strains, from the 15 patients in ICU and other wards, in their study. They reported the resistance against Linezolid is due to the presence of *cfr* but not due to G2575T mutation in 23S ribosomal RNA. They collected strains from ICU and other wards and determined the MIC using E-test and genotyped strains using PFGE followed by sequencing for presence of mutation in 23S rRNA and at last performed PCR for *cfr* gene. The results were that MIC of Linezolid was between 16 to 32 mg/L, and strains were not resistant to Vancomycin, Tigecycline and Daptomycin. Typing of strains by PFGE exposed only Linezolid resistant MRSA. The mutation which is considered responsible for Linezolid resistance was absent while the *cfr* gene was confirmed. They reported that underlying mechanism of Linezolid resistance was the presence of *cfr* gene but not the G2575T mutation.³⁶ *Cfr* was earlier identified in pSCFS1 plasmid of *Staphylococcus sciuri* isolate in 1997 from a calf in Bavaria. The calf was florfenicol treated.³⁷ To determine whether the *cfr* gene is chromosomal or plasmid borne, a study was conducted by Locke et al.³⁸ They cultured Linezolid resistant strain-1128105 (*cfr* positive MRSA), analysed its *cfr* antibiogram, extracted its plasmid and transferred it to the wild-type strain of *S. aureus* (ATCC 29213) which was not resistant to the drugs falling within the spectrum of *cfr* resistance. The outcome was positive for recapitulating the *cfr* antibiogram in transformed wildtype strain of ATCC 29213, just like the *cfr* antibiogram of 1128105 strains. The resulting (transformed) strains displayed the non-susceptibility phenotype to the drugs (falling within spectrum of *cfr* resistance) as well, and it was confirmed that it can be transferred horizontally by being plasmid borne. They also suggested that *cfr* gene environments possessed by all the three *cfr* possessing clinical isolates (1128105, CM05 & m05/0060) reported up-to 2005 were unique, emphasizing the importance of multidrug resistance *cfr* gene.³⁸ Linezolid

resistance in *S. aureus*, can be summarized in Figure 3.

The major mechanisms behind Linezolid resistant were strongly reported with presence of G2575T mutation in 23S ribosomal RNA, existence of *cfr* gene, surprisingly, a factor that suggests horizontal transfer of resistance among pathogenic strains and also L3 & L4 mutations. Some roles of homologous recombination and hypermutation has been reported as well.³⁸⁻⁴¹ In the latest findings, Linezolid resistance of *S. aureus* were associated with G2576T mutations in domain V region of 23S ribosomal RNA.¹² While according to Maarouf et al.⁴² the mutation which seems to play role in Linezolid resistance is G2603T.

Prevalence of LRSA

Antibiotic resistance among strains varies from region to region and country to country. The first clinical identification of LRSA in US was reported in 2001.^{42,43} In following years, many reports have been published relevant to LRSA presence.⁴⁴⁻⁴⁶ The criteria on which EUCAST & CLSI define Linezolid resistance in *S. aureus* is ≥ 8 mg/L MIC (Linezolid minimal inhibitory concentration). Based on this, a review was published in 2012 discussing Linezolid resistance in Staphylococcal species as emerging problem. The review was based on research survey through PubMed and EMBASE during April 2012. The terms used were based on National Library of Medicine's MeSH⁴ i.e., "resistance", "Linezolid" and "*Staphylococcus*" to retrieve articles that describe Linezolid resistant *Staphylococcus*. Then, retrieved publications were examined by title and then abstract after which required material was reviewed from the full text. The publications included short communications, original articles, correspondence, or case reports that reported required clinical isolates. To compare Linezolid resistance rates among *S. aureus* & Coagulase Negative Staphylococci, statistical analysis was performed. Two surveillance programmes, LEADER & ZAAPSS are responsible for monitoring Linezolid susceptibility among various clinically important isolates. Non-duplicate isolates from ABSSSIs and bacterial pneumonia are submitted in these programmes by participating institutes. MIC⁸ methods (Broth microdilution) were used

Table. Incidences of reported LRSA

Region	Year of report	Samples duration	Source of sample	No. / types of isolates	Isolates with linezolid resistance	Accompanying mutation or gene	Ref.
USA	2001	ND	Peritoneal Fluid	15 (MRSA)	3 (20%)	G2576T Mutations	[43]
UK	2003	ND	Body fluids and drain site swab	11 (MRSA)	3 Isolates Showed >8 mg/L MIC (20 %)	G2576T Mutation	[54]
USA	2004	ND	ND	<i>S. aureus</i> (A8761)	Positive	G2576T Mutation	[55]
Brazil	2005	2000-2002	Sputum, Throat Swab	JMI-700	Positive	G2576U Mutation	[56]
Colombia	2007	2005	Sputum	MRSA (CM05)	Positive	<i>cfr</i> Presence	[34]
USA (LEADER Program)	2007	2006	ND	2913 (<i>S. aureus</i>)	<0.1 %	ND	[57]
Germany	2008	2004-2005	Stool	Subsequently Isolated 6 MRSA from a Single Patient.	5 Strains were LRSA	G2576T Point Mutation	[58]
Japan	2009	25 Sep- 22 Dec	Sputum Blood, Stool, Catheter	Subsequently Isolated 5 MRSA from a Single Patient	Later 3 Strains were LRSA	G2576T Point Mutation	[59]
SPAIN	2010	Apr 13- June 26, 2008	Patients from ICU	NA	12 LRSA Isolates	<i>cfr</i> Presence	[44]
UK (Cystic Fibrosis)	2009	ND	Sputum & 2 LRSA Isolates	ST36 MRSA		ND	[49]
SPAIN Jun 2008	2010	Apr 2008–	ND Isolates	MRSA	12 LRSA	<i>cfr</i> Presence	[36]
Japan	2011	2006-2008	Patients from Hospitals	NA	11 LRSA Isolates	G2576T Mutation	[45]
India (Nagpur)	2012	2010-2011	Pus	51 (<i>S. aureus</i>)	12 (23.5%)	ND	[53]
Iran (Tehran)	2012	Apr- July 2011	Anterior Nares of Children	20 (<i>S. aureus</i>)	19 (95%)	15/19= <i>cfr</i> +	[60]
Spain (HUB)	2013	1999-2010	HUB Clinical MRSA Isolates	16 (MRSA)	1 (6.3%)	<i>cfr</i> Presence	[61]
Nigeria (Zaria)	2015	ND	Hands & Stuff of Hospital	57 (<i>S. aureus</i>)	0	ND	[62]
Nigeria (Amassama)	2016	ND	Anterior Nares	91 (<i>S. aureus</i>)	72 (79.1%)	ND	[63]
United States	2017	2011-2015	Leader Surveillance Programme	15177 (<i>S. aureus</i>)	<1%	<i>cfr</i> , 23S rRNA & L3 Mutation	[64]
Tanzania (Dar Es Salaam)	2017	Mar- Aug 2015	Anterior Nares	89 (<i>S. aureus</i>)	3 (3.4%)	ND	[65]

Table. Cont...

Region	Year of report	Samples duration	Source of sample	No. / types of isolates	Isolates with linezolid resistance	Accompanying mutation or gene	Ref.
Pakistan (Karachi)	2017	2012-2013	Clinical Specimens	165 (MRSA)	0	ND	[66]
Pakistan (Faisalabad)	2017	March-July	Pus	150 (34%)	51	39/50= <i>cfr</i> +	[48]
Southeast (Vietnam)	2018	Sep-Dec 2013	Nasal Cavity	205 (<i>S. aureus</i>)	4.30%	ND	[71]
Poland (Masovian)	2019	2015-2017	Clinical Specimens	112 (MRSA)	0	ND	[67]
India (Bhatinda)	Dec-2019	2016-2017	Clinical Specimens	162 (<i>S. aureus</i>)	8 (4.9%)	ND	[52]
India (Amritsar)	2019	2017-2018	Clinical Specimens	735 (<i>S. aureus</i>)	10 (1.4%)	ND	[68]
Portugal (Vila Real)	2019	ND	Ulcer Specimens from Diabetic Patients	28 (MRSA)	3 (10.7%)	<i>cfr</i> Presence	[69]
Korea	2019	Jan 2014-Dec 2018	NA	27 (MRSA)	4 (14.8%)	23S rRNA T2500A mutation	[70]

*Clinical Specimens= Blood, Urine, Pus, Sterile Body Fluids, ETT, Swabs of Ear, Throat and Vagina, Cerebrospinal Fluid and other Body Fluids. *HUB= Hospital Universitari De Bellvitge, *ND= No Data, NA= Not Applicable. USA= United States of America, UK= United Kingdom, LEADER= Linezolid Experience and Accurate Determination of Resistance, MRSA= Methicillin Resistant Staphylococcus aureus, *cfr*- Chloramphenicol-Florfenicol resistance gene, LRSA- Linezolid Resistant Staphylococcus aureus

for confirmation of Linezolid susceptibility using Clinical and Laboratory standard Institute guidelines.

Linezolid resistance documented by LEADER (according to study between 2002-2010) was in 0.05% *S. aureus* (13 out of 23077) while it was 1.4% for Coagulase negative Staphylococci, documenting increased resistance in CoNS. On the other hand, ZAAPS recognized 10 Linezolid resistant CoNS⁹ and only one strain of LRSA. ZAAPS (2004-2010) yielded overall rate 0.14% among 8122 Staphylococci. In addition to this study, another study is mentioned in which researchers reviewed 22 publications reporting approximately 65 cases of LRSA and 28 publications reporting 351 cases of Linezolid resistant CoNS^{10, 46}

In Turkey, an experiment revealed that no Linezolid resistant strain was found in their research. The drive of research was to explore the phenotypic resistance of MRSA and MSSA (321 & 195 respectively) strains to Linezolid, Macrolide,

Streptogramin B, Lincosamide, and Ketolid. Kirby Bauer test was applied to determine MLSB phenotype and susceptibility to other antibiotics. They found that 0.4% of isolates were resistant to quinupristin half pristin, 48% to Clindamycin, 54.6% to erythromycin, 58.7% to spiramycin, 55% to azithromycin, 34.7% to telithromycin. They could not find any strain resistant to Linezolid.⁴⁷ While on the other hand, a study conducted in a region of Pakistan (Faisalabad) reported the identification of 34% isolates were LRSA (*cfr* positive) out of 150 *S. aureus* isolates from pus samples (of skin, ears and wounds) of patients from local hospital⁴⁸ indicating the variable prevalence of LRSA comparing to study explained before. Thus, the prevalence of antibiotic resistance in micro-organisms differs with respect to the regions as various studies just like in Turkey reported no strain of LRSA. The short review about some worldwide reported LRSA for 2 decades is shown in Table. The other reported factors for variable Linezolid resistance

in pathogen are drug dosage (low/high dosage),⁴⁹ and cystic fibrosis.⁵⁰ Another study on CF aspect in Cleveland, Ohio reported that more than 10 percent CF patients receiving Linezolid treatment were developing resistance.⁵¹ Most of the LRSA strains were isolated from soft skin tissues in their study. They used disc diffusion method for determination of antimicrobial susceptibility. Eight strains were reported LRSA out of 162 *S. aureus* strains. Out of them five isolates were from pus, two from throat, ear, and vaginal swab, and last one was from blood. Kaur et al.⁵² showed high prevalence of LRSA in pus which was 62.5%. Azhar et al.⁴⁸ showed 34 % and the other conducted by Thool et al.⁵³ reported 23.5 % prevalence of LRSA in pus as given in Table.^{34,36,43-45,48,49,52-71} It can be seen from the data that going through the countries (Geographical region), the majority of LRSA was from Europe and Asia. The data on Linezolid resistance in *S. aureus* and the cause of Linezolid resistance (if any) is not sufficiently reported among southeast Asian countries.

Ongoing Challenges of Linezolid Resistance and Possible Solutions

Emergence of resistance in *S. aureus* against Linezolid is utterly posing a risk to antibiotic treatment. As Linezolid is considered the best therapy in case of Vancomycin resistance in *S. aureus* infections but in an antimicrobial study conducted on bacterial samples isolated from the 518 pus samples of wounded patients: who presented at Ghurki Trust Teaching Hospital Lahore, Pakistan from January 2019 to January 2020, indicated that microbial sensitivity of Vancomycin was higher than the Linezolid.⁷² This can be due to irrational prescription of Linezolid without testing the exact microbial strain from patient who are being recommended antibiotics. In contrast, it is reported that reduced Linezolid dosing schemes in elderly patients should be considered as low risk of treatment failure, ultimately increasing the effectiveness of the drug.⁷³

Since the incidences of resistance against Linezolid in *S. aureus* are being reported, the researchers are also in a race of dealing such challenge. For example, in order to enhance Linezolid susceptibility in *S. aureus* strain, antibiotic adjuvants are in study. Honey is also

considered, by many people, a natural remedy of several diseases. It is used as food as well as medicine in many cultural groups because of its therapeutic properties. For example, honey is considered beneficial for ulcer, burns, cataracts, and wounds. Depending on origin, honey has been branded into different types.⁷⁴ Evidence for Manuka Honey (Mono-floral honey isolated from nectar of manuka tree, New Zealand) to elevate antibiotic sensitivity in *S. aureus* are reported.^{75,76} MGO (methylglyoxal) is reported the active gradient of manuka honey that supports the increased intracellular accumulation of Linezolid in *S. aureus*.⁷⁷ Additionally, the next to Linezolid, investigational effective antibacterial drug against LRSA strains was a new (2nd generation) oxazolidinone, TR-700 (Tedizolid). Its potency comparing with Linezolid is reported around 2 to 8-fold,⁷⁸ against multidrug resistant Gram-positive bacteria. Tedizolid was approved by FDA in June 2014 for treatment of acute skin infections caused by Gram-positive bacteria.⁷⁹

Ever increasing issue of antibiotic resistance cannot be halted but we can adopt preventive measures to reduce the chance of resistance acquisition in microbial strains such as proper and regular sterilization of surgery equipment and other stuff in hospitals as one reason of LRSA emergence is horizontal gene acquisition as cited above and its outbreak in nosocomial infections. Secondly, the subtherapeutic dosage of drug is also responsible for Linezolid resistance in *S. aureus*; therefore, proper identification of pathogen is recommended before the use of any antibiotic drug based on the sample/strain identification from individual and not on basis of common epidemiology in the area. The blind use of antibiotic is one of the major causes of emergence of antibiotic resistant strains. Finally, the pressure on Linezolid therapy due to *S. aureus* resistance can be dealt by the understanding of epidemiology of antibiotic resistance, this knowledge will help to pick up preventive strategies to reduce existing resistance and to prevent the appearances of new resistant strains.

CONCLUSION AND PERSPECTIVES

A continuous increasing resistance in *S. aureus* can cause different kinds of infections on

skin and tissues in addition to other organs. This pathogen has many virulence factors including TSST-1 and a large disease index. The antibiotic treatment ranges from Penicillin, Methicillin, Vancomycin and Linezolid depending on what kind of strain is responsible for the infection. However, the pathogen had shown resistance against each of the aforementioned antibiotics. The intimidating fact is the resistance against Linezolid. Reported incidences of Linezolid resistance has troubled the success of antibiotic therapy as it was a novel synthetic drug. It was supposed that no resistance gene could exist for Linezolid in *S. aureus* as it was synthetic in nature, but this pathogen has shown resistance despite of lacking resistance gene in its genome through mutational processes and also through the acquisition of resistance gene from other bacteria. The problem of resistance of one bacterial specie against many drugs has troubled the antibiotic treatment choice. It has become important to check LRSA before using Linezolid drug against MRSA and VRSA (Both showed resistance against Linezolid) as its injudicious use will not only cause further drug resistance but also fail the treatment of bacterial infection. The incidences of LRSA prevalence as shown in Table that it is 0% in one report and 95% in another, indicate that LRSA prevalence vary from one place to another which may be due to different mechanism of resistance development or acquisition, or it may be due to irrational prescriptions of the concerned drug. Its prevalence is uncertain, and reason could be the lack of sanitization/sterilization in hospitals, thus resulting to the possibility of horizontal gene transfer. In order to prevent the incidences of LRSA, precautionary measures must be adopted such as sterilization of equipment that are used in hospitals especially during surgery, proper sanitization, public awareness of antibacterial resistance and most importantly the appropriate drug prescription and use.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

HR, NH and MB conceptualize the review. HR, NH, MUS and GA did the literature screening and data curation. HR, NH, MUS, HMNI, GA and MB wrote original draft. HMNI and MB did the reviewing and editing of the manuscript. All the authors have read and approved it for publication.

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DATA AVAILABILITY

All data sets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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