Original Article

Antibacterial Effects of Grapes Seed Extract on Methicillin Resistant Staphylococcus Aureus and Extended Spectrum Beta Lactamase Producing Escherichia Coli and its Comparison with Linezolid and Meropenem

Ali Nawaz Bijarani[1], Muhammad Owais Ismail[3], Zahida Memon[3], Faisal Afridi[4], Shabana Qabulio[2] and Akhtar Ali[3]

ABSTRACT

Objective: Antibacterial effects of grapes seed extract on methicillin resistant staphylococcus aureus and extended spectrum beta lactamase producing escherichia coli and its comparison with linezolid and meropenem.
Study Design: It is a Pre-Clinical (in-vitro) study
Place and Duration of Study: This study was conducted at the Ziauddin University, Karachi from January 2020 to October 2020 for a period of 10 months.
Materials and Methods: Patients age were 10 to 80 years, both genders, showing signs and symptoms of bacterial infections were selected. All clinical samples were collected in the form of pus, urine, blood, tracheal aspirations, patients admitted in surgical and medical wards, intensive care units and outdoor patients, who were attending clinics. All these samples were transported to Clinical Microbiology Laboratory, Ziauddin hospital, North Nazimabad campus and then culture and sensitivity test were performed there. Sample showing double growth and contamination on agar plates were excluded from study.
Results: Total 80 samples were collected. 40 samples were Methicillin Resistant Staph Aureus (MRSA) and 40 samples were Extended Spectrum Beta Lactamase Producing Escherichia Coli (ESBL E Coli). Female to male samples ratio of 1.35:1. Methicillin Resistant Staph Aureus commonly found in pus swab 15(37.5%) and 21(52.5%) ESBL Producing E Coli found in urine samples. Anti-bacterial activity of Grapes seed extracts observed against Methicillin Resistant Staph Aureus at different levels of concentration 37(92.5%), 37(92.5%), 37(92.5%), 40(100%) and 40(100%) at different dilutions at 20 mg/ml, 30 mg/ml, 40 mg/ml and 40 (100%) respectively. While less resistance was observed of Methicillin Resistant Staph Aureus at different concentration level of Grapes seed extracts. More resistance was observed of Grapes seed extracts against ESBL E coli at different concentration reported about 97-100%. While sensitivity of ESBL E coli was observed only in 3 sample at different concentration level of Grapes seed extracts about 2.5%. Compare the results of sensitivity of GSE with linezolid and meropenem against ESBL E coli and found significant p value <0.05.
Conclusion: The grape seed extract has shown excellent antimicrobial activity against methicillin resistant Staph aureus and resistant strains of ESBL producing E. coli.
Key Words: Grapes seed extract, Methicillin Resistant Staphylococcus Aureus, Escherichia coli

Citation of article: Bijarani AN, Ismail MO, Memon Z, Afridi F, Qabulio S, Ali A. Antibacterial Effects of Grapes Seed Extract on Methicillin Resistant Staphylococcus Aureus and Extended Spectrum Beta Lactamase Producing Escherichia Coli and its Comparison with Linezolid and Meropenem. Med Forum 2021;32(9):28-32.

INTRODUCTION

[1.] Department of Pharmacology / Pathology[2], Shaheed Mohtarma Benazir Bhutto Medical College, Liyari, Karachi.
[3.] Department of Pharmacology / Microbiology[4], Ziauddin University Karachi.

Correspondence: Dr. Ali Nawaz Bijarani, Demonstrator, Department of Pharmacology, Shaheed Mohtarma Benazir Bhutto Medical College, Liyari Karachi.
Contact No: 0300-2639709
Email: alibijarani@gmail.com

Received: April, 2021
Accepted: July, 2021
Printed: September, 2021

The ruthless abuse of antibiotics is continually causing an increase in the resistant bacterial strains to one or more than one drug which we name multidrug resistant (MDR) bacteria.[1] The discovery of methicillin-resistant Staphylococcus aureus (MRSA) which is resistant to certain penicillin and cephalosporins along with methicillin in 1960s can be quoted as one other example of MDR bacteria.[2] The resistance and

sensitivity of the organism towards one or more drugs changes the scenario of treatment and over all outcome of the treatment of patient and the MDR definitely have a solid negative impact on the mortality rate of the patients as compared to the single drug resistant or susceptible strains.[3] Therefore, in many fields including tissue engineering ,biotechnology and microbiology a continues endeavor is being carried out over discovery of new elements/substances for targeting MDR bacteria.[4] Natural and plant based products have old roots in the basis of treatment in various infections[5]. These natural products include the antibacterial effect of cranberry extract, honeydew, black pepper extract, honey, grape seed extract (GSE), and coral Hibiscus extract, which has also been authenticated by many studies.[6] In recent days natural/plant based derivatives for various bacterial infections are easily available in the market giving us a fair and clear idea that natural products are very easily accepted by the patients and the body with no or least resistance as compared to the laboratory based synthetic products.[7] In this study, we studied the effect of grape seed extract on Methicillin Resistant Staphylococcus aureus and Extended Spectrum beta lactamase producing Escherichia coli and its comparison with Linezolid and Meropenem. From the screening test, only GSE showed good antibacterial effect.[8] Hence, the aim of this study was to access the antibacterial activity of GSE towards MDR bacteria such as Methicillin Resistant Staphylococcus Aureus and Extended Spectrum beta lactamase producing Escherichia coli and its comparison with Linezolid and Meropenem as they cause severe nosocomial infection, and look into the exact mechanism of the antibacterial effect of Grape Seed Extract[9].

MATERIALS AND METHODS

It is a Pre-Clinical (in-vitro) study conducted in Ziauddin University from January 2020 to October 2020. All samples were collected from Ziauddin University. Patients age were 10 to 80 years, both genders, showing signs and symptoms of bacterial infections were selected. All clinical samples were collected in the form of pus, urine, blood, tracheal aspirations, patients admitted in surgical and medical wards, intensive care units and outdoor patients, who were attending clinics. All these samples were transported to Clinical Microbiology Laboratory, Ziauddin hospital, North Nazimabad campus and then culture and sensitivity test were performed there. Sample showing double growth and contamination on agar plates were excluded from study.

Herbal Extraction and Authentication: Grapes Seed was purchased from commercial market, Karachi and stored at room temperature. Authentication (95627) were done from Botany department, Karachi University. Extraction procedure were done at Pharmacognosy department, ZU Karachi. The final preparation was stored at normal room temperature and used for experimental work.

Preparation of Grapes Seed Extracts: Fully ripe grapes were purchased from a commercial market, Karachi. The grapes were crushed and seeds separated. The seeds were washed well using clean water and dried in oven at 60oC. The seeds then powdered in a grinder. 20g of grape seed powder was added to a conical flask containing 100 ml of ethanol and stirred well. The flask was left aside for 48 hours and occasionally stirred. The content of flask was filtered through Whatman and also evaporated to dryness in oven a 50oC. After the grape seed extract had been obtained, different concentrations of grape seed extract (20, 30, 40, 50 and 60 mg/ml) were prepared by mixing the grape seed powder with dimethyl sulfoxide (DMSO) and stored at 4°C in air tight bottles for further studies.

RESULTS

Total 80 samples were collected. 40 samples were Methicillin Resistant Staph Aureus (MRSA) and 40 samples were Extended Spectrum Beta Lactamase Producing Escherichia Coli (ESBL E Coli). The 80 samples of specimens were processed in culture plates and the pathogens were isolated and identified by standard biochemical tests. Out of 80 samples included in this study 46(57.5%) were female and 34(42.5%) samples were male; female to male samples ratio of 1.35:1. There was wide variation of age ranging from a minimum of 10 year to 79 years. The mean age was 45.71±11.83 years (Table No.1). In our study Methicillin Resistant Staph Aureus commonly found in pus swab 15(37.5%) and 21(52.5%) ESBL Producing E Coli found in urine samples (Table No.1). Most Commonly MRSA 13(32.5%) was found in indoor specially in surgical department, while ESBL producing E coli 12(30%) were found in medical ward admitted patients (Table No.1). In our study, we observed meropenem 100% sensitive against Extended Spectrum Beta Lactamase Producing Escherichia coli sample as compare to Methicillin Resistant Staphylococcus Aureus 5(15%) samples. While resistance was observed in Methicillin Resistant Staphylococcus aureus 34 (85% samples), while Linezolid 37(92.5%) were sensitive in Methicillin Resistant Staphylococcus aureus. While resistance was observed in Extended Spectrum Beta Lactamase Producing Escherichia coli 40 (100%) samples. (Chart No.1). In our study, best anti-bacterial activity of Grapes seed extracts observed against Methicillin Resistant Staph Aureus at different levels of concentration 37(92.5%), 37(92.5%), 37(92.5%), 40(100%) and 40(100%) at different dilutions at 20 mg/ml, 30 mg/ml, 40 mg/ml and 40 (100%) respectively. While less resistance was observed of Methicillin Resistant Staph Aureus at different

concentration level of Grapes seed extracts. (Chart No.2).

Our study shows more resistance were observed of Grapes seed extracts against ESBL E coli at different concentration reported about 97-100%. While sensitivity of ESBL E coli was observed only in 3 sample at different concentration level of Grapes seed extracts about 2.5% (Chart No.3). Compare the results of sensitivity of GSE with linezolid and meropenem against ESBL E coli and found significant p value <0.05. In our study more resistance activity was observed of Grapes Seed Extract against ESBL E coli at different concentration. While sensitivity of ESBL producing E coli were observed only in 3 sample at different concentration level of Grapes seed extracts (Chart No.4).

Table No.1: Variable

Variable	MRSA	ESBL E Coli
Gender		
• Male	21(52.5%)	15(37.5%)
• Female	19(47.5%)	25(62.5%)
Age in years		
• 10-30 years	7(17.5%)	9(22.5%)
• 31-50 years	11(27.5%)	10(25%)
• 51-70 years	16(40%)	15(35%)
• >71 years	6(15%)	7(17.5%)
Samples Sources		
• Blood	9(22.5%)	8(20%)
• Pus	15(37.5%)	5(12.5%)
• Tracheal Asp	11(27.5%)	6(15%)
• Urine	5(12.5%)	21(52.5%)
Department		
• Surgical ward	13(32.5%)	5(12.5%)
• ICU/CCU	11(27.5%)	9(22.5%)
• Medical Ward	6(15%)	12(30%)
• Gynae/Obs	5(12.5%)	6(15%)
• Outpatients Department	5(12.5%)	8(20%)

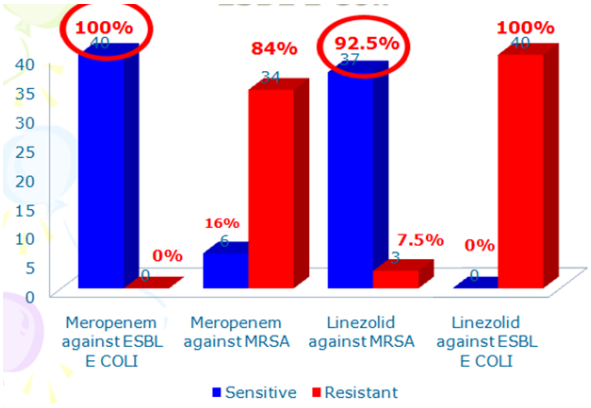

Chart No.1: Antibiotic susceptibility of MRSA and ESBLA E Coli

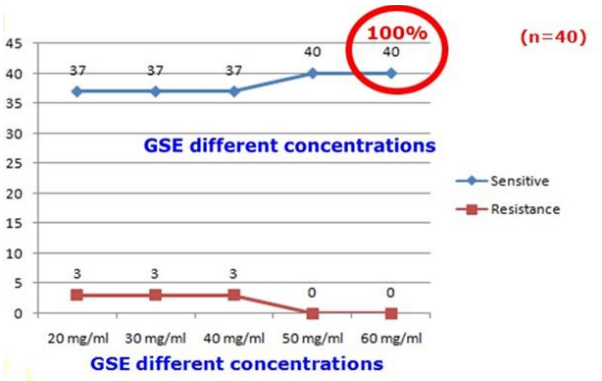

Chart No.2: Sensitivity of Anti-bacterial activity of GSE against MRSA

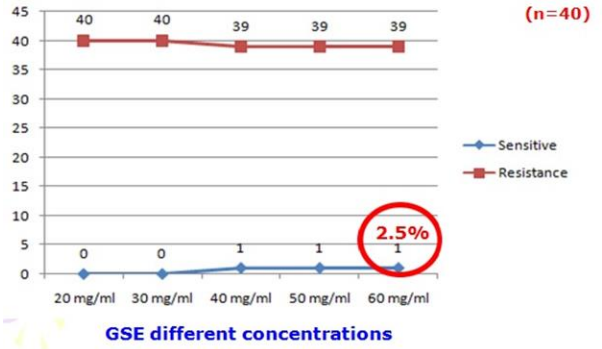

Chart No.3: Sensitivity of Anti-Bacterial activity of GSE agaisnt ESBL E Coli

	Plant EXTRACT	STANDARD DRUGS	P VALUE
ESBL E Coli	GSE 60mg/ml 2.5%	Meropenem 100%	<0.05
	GSE 60mg/ml 2.5%	Linezolid 0%	Not significant
MRSA	GSE 60mg/ml 100%	Meropenem 16%	<0.05
	GSE 60mg/ml 100%	Linezolid 92.5%	Not significant

Chart No.4: Comparison of Sensitivity of GSE with linezolid and meropenem against Anti-Bacterial activity of GSE agaisnt ESBL E Coli

DISCUSSION

Multi-drug resistance (MDR) is becoming common day by day, because of the undue usage of antibiotics by the quakes and local general practitioners leading to the new pandemics.[10] The development of antibiotics since 1960's provided the sign of relief in the treatment of many emerging antimicrobial infections.[11] But the excessive and undue usage of these drugs is almost going to lead us to facing pre- antibiotic era due to development resistance.[12] The development of drug resistance is found against one or more than one drug called multi drug resistance MDR favoring the need of

further studies to be carried out to find out the new substances which can help treating the MDR bacterial infections.[13] Methicillin resistant staphylococcus aureus and beta- lactam resistant E. coli are one of the most suitable examples in regard to MDR bacteria.[14] In our study we have tried to focus on the plant based Grape seed extract for the treatment as it has ability to interfere the resistance and can also increase the efficacy of β-lactam agents.[15] The basic aim of this study was to find out that wither the grape seed extract can be used for treating beta lactam resistant E. coli and MRSA and its comparison with linezolid and meropenem used for the same purpose.[16]

In this study, we extracted the grape seed extract by standard method of extraction and used it in 80 patients with high female to male ratio as 1.35:1, which is almost similar to various previous studies[17]. The mean age of MDR bacterial infections found in our study was 45.71±11.83 years from the range of 10-79 years which is similar to as elicited by Dale, A., Pandey at al.[18] In our study we reported MRSA isolation from pus and wound samples, while ESBL E.coli from the urine samples which is also supported by Beytur, A., Yakupogullari at al.[19] Most Commonly MRSA 13(32.5%) was found in indoor specially in surgical department, while ESBL Producing E coli 12(30%) were found in medical ward admitted patients, this kind of specific association of the organisms to medical and surgical indoors is also reported in certain previous studies.[20]

Oliva, A., Costantini at al. in their study reported ESBL producing E. coli to be more sensitive to meropenem similar to the results of our study in this regard stated as Meropenem is 100% sensitive in Extended Spectrum Beta Lactamase Producing Escherichia Coli and more resistant to Methicillin Resistant Staphylococcus aureus 34(85% samples), while Linezolid 37(92.5%) were sensitive in Methicillin Resistant Staphylococcus aureus and more resistant to ESBL producing E. coli 40(100%) according to our study.[21] The concentration of the natural product extract (grapes seed extract) also matters a lot as according to this study, best anti-bacterial activity of Grapes seed extracts was obtained at different levels of concentration 37(92.5%), 37(92.5%), 37(92.5%) samples were 20 mg/ml, 30 mg/ml, 40 mg/ml and 40 (100%) each were sensitive at 50mg/ml and 60mg/ml respectively. While less resistance was observed of MRSA at different concentration level of Grapes seed extracts.[22] About 97-100% resistance was reported for Grapes seed extracts against ESBL E coli at different concentration levels. which is somewhat in accordance with WHO 2008.[23]

Hence the plant based natural extracts like grape seed extract can be considered as a good alternative of the antibiotic therapy according to our study and can prove to be a source of decrement in the economic load of antibiotic usage. Furthermore, we stress the use of antibiotics according to the respective doctor's prescription and after the culture and sensitivity report of the patient to reduce the chances MDR bacterial production. AMR and expensive antibiotics are becoming a significant economic burden[24]. We support the use of grape seed extract for the treatment of MDR bacterial infections according to the results of our study.

CONCLUSION

The grape seed extract has shown excellent antimicrobial activity against methicillin resistant Staph aureus and resistant strains of ESBL producing E. coli in comparison to linezolid and meropenem and so it can be used as a potential substance to treat the MDR bacterial infections alone.

Author's Contribution:

Concept & Design of Study:	Ali Nawaz Bijarani
Drafting:	Muhammad Owais Ismail, Zahida Memon
Data Analysis:	Faisal Afridi, Shabana Qabulio, Akhtar Ali
Revisiting Critically:	Ali Nawaz Bijarani, Muhammad Owais Ismail
Final Approval of version:	Ali Nawaz Bijarani

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Magiorakos AP, Srinivasan A, Carey RT, Carmeli Y, Falagas MT, Giske CT, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268–281.
2. Anudit C, Kooltheat N, Potup P, Sranujit RP, Usuwanthim K. Nosocomial infection of multidrug-resistant Acinetobacter baumannii in Thailand. Am J Infect Control 2016;44:1161–63.
3. Vardakas KZ, Rafailidis PI, Konstantelias AA, Falagas ME. Predictors of mortality in patients with infections due to multi-drug resistant Gram negative bacteria: The study, the patient, the bug or the drug? J Infect 2013;66:401–14.
4. Qiao S, Wu D, Li Z, Zhu Y, Zhan F, Lai H, et al. The combination of multi-functional ingredients-loaded hydrogels and three-dimensional printed porous titanium alloys for infective bone defect treatment. J Tissue Eng 2020;11.
5. Da Silva BL, Caetano BL, Chiari-Andréo BG, Pietro RCLR, Chiavacci LA. Increased antibacterial activity of ZnO nanoparticles: Influence of size and surface modification. Colloids Surf. B Biointerfaces 2019;177:440–7.

6. Lakkim V, Reddy MC, Pallavali RR, Reddy KR, Reddy CV, Bilgrami AL, et al. Green synthesis of silver nanoparticles and evaluation of their antibacterial activity against multidrug-resistant bacteria and wound healing efficacy using a murine model. Antibiotics 2020;9:902-11.
7. Tang S, Zheng J. Antibacterial activity of silver nanoparticles: Structural effects. Adv Healthc Mater 2018;7:1701503.
8. Yang X, Yang J,Wang L, Ran B, Jia Y, Zhang L. Pharmaceutical intermediate-modified gold nanoparticles: Against multidrug-resistant bacteria and wound-healing application via an electrospun scaffold. ACS Nano 2017;11:5737–45.
9. Abdallah EM, Koko WS. Medicinal plants of antimicrobial and immunomodulating properties. In Antimicrobial Research: Novelbioknowledge and Educational Programs; Formatex Research Center: Badajoz, Spain 2017;127–139.
10. Escherichia coli Infections - Infections - MSD Manual Consumer Version. [online] Escherichia coli Infections. Available at: https://www.msdmanuals.com/en-gb/home/infections/bacterial-infections-gramnegative-bacteria/escherichia-coli-infections [Accessed 26 Sep. 2019].
11. Chambers HF, DeLeo FR. Waves of resistance: Staphylococcus aureus in the antibiotic era. Nature Reviews Microbiol 2009;7(9):629-41.
12. Cooper K, Lamagni T, Harrington P, Wloch C, Hopkins S. Surveillance Of Surgical Site Infections In NHS Hospitals In England. 2019. [online] Assets.publishing.service.gov.uk. Available at: [Accessed 9 November 2020].
13. Pangilinan C, Dhayalan A, Gracilla D, Dela Peña R, Malison M. Phytochemical constituents and antimicrobial activity of the ethanol and chloroform crude leaf extracts of Spathiphyllum cannifolium (Dryand. ex Sims) Schott. J Pharmacy And Bioallied Sciences 2018;10(1):15-9.
14. Renwick M, Brogan D, Mossialos E. A systematic review and critical assessment of incentive strategies for discovery and development of novel antibiotics. J Antibiotics 2016;69(2):73-88.
15. Roser M, Ortiz-Ospina E, Ritchie H. Life Expectancy 2020. [online] Our World in Data. Available at: [Accessed 11 December 2019].
16. Dale AP, Pandey AK, Hesp RJ, Belogiannis K, Laver JR, Shone CC, et al. Genomes of Escherichia coli bacteraemia isolates originating from urinary tract foci contain more virulence-associated genes than those from non-urinary foci and neutropaenic hosts. J Infection 2018;77(6):534-43.
17. Sakr A, Brégeon F, Mège JL, Rolain JM, Blin O. Staphylococcus aureus nasal colonization: an update on mechanisms, epidemiology, risk factors, and subsequent infections. Frontiers Microbiol 2018;9:2419-21.
18. Beytur A, Yakupogullari Y, Oguz F, Otlu B, Kaysadu H. Oral amoxicillin-clavulanic acid treatment in urinary tract infections caused by extended-spectrum beta-lactamase–producing organisms. Jundishapur J Microbiol 2015;8(1): e13792.
19. Taylor T, Unakal C. Staphylococcus aureus. [online] Ncbi.nlm.nih.gov. Available at 2020: [Accessed 11 November 2019].
20. Who.int. Antimicrobial resistance 2018. [online] Available at: https://www.who.int/en/news-room/fact-sheets/detail/antimicrobial-resistance [Accessed 25 Sep. 2019].
21. Oliva A, Costantini S, De Angelis M, Garzoli S, Božović M, Mascellino MT, et al. High potency of Melaleuca alternifolia essential oil against multi-drug resistant gram-negative bacteria and methicillin-resistant Staphylococcus aureus. Molecules 2018;23(10):2584-90.
22. World Health Organization. WHO Guidelines for Safe Surgery: 2009. Geneva: World Health Organization; 2009.p.44-46.
23. Zeng X, Lin J. Beta-lactamase induction and cell wall metabolism in Gram-negative bacteria. Frontiers in microbiology. 2013 May 22;4:128-31.
24. World Health Organization. WHO Guidelines for Safe Surgery: 2009. Geneva: World Health Organization 2009.p.44-46.