Maqbul et al., *J. Pure Appl. Microbiol.,* **14**(**1**), 255-260 | March 2020
Article 5994 | https://doi.org/10.22207/JPAM.14.1.26

Print ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Comparison of e-test Values for Standard Antibiotics and Conventional Antimicrobial Assay Values for Ethanoic Acids against Nosocomial Multidrug-resistant *Pseudomonas aeruginosa*

Muazzam Sheriff Maqbul[1], Ali Mohamed Alshabi[2], Aejaz A. Khan[3], S.M. Shakeel Iqubal[3]*, Tasneem Mohammed[3], Ibrahim Ahmed Shaikh[4], Areej Dawoud[3], Uday M. Muddapur[5], Mohammed Shahid Hussain[6] and S.K. Singh[7]

[1]Faculty of Microbiology and Immunology, Ibn Sina National College of Medical Sciences, Al Mahjar Street: 31906, Jeddah 21418, Kingdom of Saudi Arabia. [2]Department of Clinical Pharmacy, College of Pharmacy, Najran University, Najran, Saudi Arabia. [3]Department of General Science, Ibn Sina National College of Medical Sciences, Al Mahajar Street: 31906, Jeddah 21418, Kingdom of Saudi Arabia. [4]Department of Pharmacology, College of Pharmacy, Najran University, Najran, Saudi Arabia. [5]Department of Biotechnology, KLE Technological University, BVB Campus, Hubballi, 580031, India. [6]Department of Orthodontics and Dentofacial Orthopedics, M. A. Rangoonwala Dental College, Pune, Maharashtra, India. [7]Department of Chemistry, GGV (Central University), Bilaspur (C.G) - 495 009, India.

Abstract

The present study aimed to determine the susceptibility of *Pseudomonas aeruginosa* strains isolated from patients with nosocomial infections to standard synthetic chemical antibiotics and organic ethanoic acids derived from local produce. The minimum inhibitory concentrations (MIC) of the standard synthetic antibiotics determined from standard e-test results and antibiotic sensitivity tests showed many multidrug-resistant strains among the isolates. We compared the susceptibility of these strains to organic ethanoic acids derived from different sources using standard microbiological assays. All strains of *P. aeruginosa* isolated from the patients were susceptible to the organic ethanoic acids with a satisfactory MIC and minimum bactericidal concentrations. Therefore, organic ethanoic acids were more effective against *P. aeruginosa* than standard synthetic antibiotics.

Keywords: Antimicrobial activity, *Pseudomonas aeroginosa,* ethanoic acid, Nosocomial infection, Immuno suppressed, Vinegar

***Correspondence:** shakeeliqubal@gmail.com; +966-570158198

(Received: December 13, 2019; accepted: March 18, 2020)

Citation: Muazzam Sheriff Maqbul, Ali Mohamed Alshabi, Aejaz A. Khan, S.M. Shakeel Iqubal, Tasneem Mohammed, Ibrahim Ahmed Shaikh, Areej Dawoud, Uday M. Muddapur, Mohammed Shahid Hussain and S.K. Singh, Comparison of e-test Values for Standard Antibiotics and Conventional Antimicrobial Assay Values for Ethanoic Acids against Nosocomial Multidrug-resistant *Pseudomonas aeruginosa*, *J. Pure Appl. Microbiol*., 2020; **14**(**1**):255-260. https://doi.org/10.22207/JPAM.14.1.26

INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative motile bacterium that is a major cause of community-acquired, and hospital-borne nosocomial infections[1,2]. The major concern about nosocomial infections with *P. aeruginosa* is that > 10% of infections are caused by multidrug-resistant species[1,3,4] that arise through antibiotic abuse. *Pseudomonas* is only one among many bacterial species that have become resistant to multiple antibiotics, and the most resistant strain is *P. aeruginosa*[5]. Gessard isolated *P. aeruginosa* from green pus in 1882 and since then it has been studied in detail, especially with regard to nosocomial infections[1]. The characteristic virulence factors of this bacterium are the exopigments pyoverdin and pyocyanin, which are potentially involved in the occurrence of these infections[1]. *Pseudomonas aeruginosa* causes versatile infections in humans, especially when immunosuppressed, and it is categorized as the most important bacterial cause of infections acquired during prolonged hospitalization. This bacterium causes frequent infections due to its natural adaptability and abundance, and it has emerged as the most important species with which to analyze multidrug resistance[1,6]. Here, we isolated multidrug-resistant strains from clinical specimens derived from patients with nosocomial infections, then compared the susceptibility of these strains to commercial synthetic antibiotics and to ethanoic acids prepared from organic apple cider, sugar molasses, dates, grapes, and grains using conventional standard microbiological techniques[4,7,8,9]. *Pseudomonas aeruginosa* ATCC27853 was the standard control strain. Because ethanoic acids contains carboxylic acids that control the formation of *P. aeruginosa* biofilm, they should help to suppress infection with this bacterium[6,8,9].

MATERIALS AND METHODS

All chemicals, reagents, and media components for this study were purchased from Sd Fine Chem Ltd, (Kolkata, India) Loba Chemie PvT., Ltd. (Mumbai, India), HiMedia (Mumbai, India), *bioMérieux SA.,* (Marcy l'Etoile, France or Sigma Aldrich Corp. (St. Louis MO, USA).

Isolation and purification of *P. aeruginosa*

Green pus, green catheters, urine, greenish ventilators, abscesses, throat swabs, nasal swabs, aspirates and collected from patients with nosocomial infections, and samples collected at surgery were processed using standard aseptic microbiological techniques. The samples were streaked onto plates containing cetrimide agar, a selective and differential medium, and incubated overnight at 37°C to allow *P. aeruginosa* to secrete exopigments. The isolates were identified and purified based on positive oxidase tests. The authenticity of the standard *P. aeruginosa* ATCC 27853 strain was confirmed in the same manner[5,10,11].

Antimicrobial susceptibility tests

The antimicrobial susceptibility of the clinical isolates and the standard strain to standard synthetic chemical antibiotics was assessed using rapid e-tests[4,5,7], Briefly, the isolates were inoculated onto separate plates containing Mueller-Hinton agar and standard e-test plastic strips were infused with each antibiotic and incubated with the isolates at 37°C overnight to develop zones and ellipses. The interaction of the ellipse was taken as the minimum inhibitory concentration (MIC), whereas the zone indicated the susceptibility of the antibiotic to the bacterium. Conventional standard was employed to observe The susceptibility of the *P. aeruginosa* isolates and the standard strain to five organic ethanoic acids was assessed by the standard diffusion method using Kirby-Bauer discs. Briefly, the isolates were inoculated separately on Mueller-Hinton agar plates with the infused discs for 24 h at 37°C to form zones, indicating the sensitivity of the bacterium toward the organic ethanoic acids. The MIC and minimum bactericidal concentration (MBC) reflecting the ability of ethanoic acid to kill the bacterium were estimated using the standard tube dilution method. Briefly, the isolates were separately inoculated into ethanoic acids diluted in peptone water, and incubated for 24 h at 37°C. The absence of turbidity indicated the sensitivity of the bacterium to the test agent. The last dilution with turbidity determined the MIC value of the test agent against the bacterium.

The MBC was determined by inoculating each dilution of MIC onto separate agar plates

for each isolate[5,10,11,12], then incubating them for 24 h at 37°C. The first dilution with no growth defined the MBC of the acid toward the bacterium *Pseudomonas aeruginosa* ATCC278.

RESULTS AND DISCUSSION

The results obtained from the e-tests of the susceptibility of *P. aeruginosa* to standard synthetic chemical antibiotics compared with organic ethanoic acid showed that none of the clinical *P. aeruginosa* isolates were resistant to organic ethanoic acid, whereas all the clinical isolates were resistant all the standard synthetic antibiotics tested. The best and worst antibiotic zone diameters for the standard synthetic chemical antibiotics against the clinical isolates

Table 1. Comparison of *Pseudomonas aeruginosa* susceptibility to standard antibiotics according to e-test zone diameters (mm).

Specimen	Antibiotics														
	AK	PM	TZ	TX	GM	MP	NI	PTC	TC	TLC	TM	TS	S	I	R
Green pus	2 R	3 R	2 R	4 R	2 R	20 S	18 S	2R	19S	21 S	12 I	20S	5	1	6
Green cath	131	2 R	3 R	2 R	4R	12 I	20 S	22S	20S	23S	21S	20S	6	2	4
Urine	4R	3 R	2 R	4 R	2R	20S	20 S	20S	20S	20S	10I	10I	5	2	5
GV	5R	2 R	2 R	4 R	2R	10I	20 S	21S	21S	20S	10I	11I	5	3	4
Abscess	4R	3 R	2 R	4 R	2R	20 S	20 S	10 I	10I	21 S	20 S	20S	5	2	5
Throat swabs	2 R	4 R	2R	21S	20S	2 R	4 R	22S	20S	20S	19S	18S	7	0	5
Aspirates	2 R	4 R	2R	4 R	2R	9I	11I	21S	20S	20S	4R	2R	3	2	7
Wound	4 R	2 R	4 R	2 R	9 I	11 I	6 R	20S	20S	2 R	4 R	2 R	2	2	8
Nasal swabs	2 R	22 S	20 S	10 I	20S	20 S	21S	20S	23S	20S	4R	3 R	8	1	3
Surgical sources	4R	3 R	2 R	4 R	2R	4 R	2R	9 I	11I	21S	20S	20S	3	2	7
ATCC 27853 Control	11I	20 S	9I	11I	22 S	20 S	10I	20S	20S	21S	20S	23S	8	4	0
Mean Zone value for all the specimens	7.5	12	11	11.5	11	11	11.5	12	16.5	12.5	12.5	12.5	5	2	4

I, intermediate susceptibility; R, resistant; S, susceptible.

Table 2. Comparison of *Pseudomonas aeruginosa* susceptibility to standard antibiotics determined as minimum inhibitory concentrations in e-tests.

Specimen	Antibiotic MIC (µg/mL)											
	AK	PM	TZ	TX	GM	MP	NI	PTC	TC	TLC	TM	TS
Green pus	1	1	1	0.25	0.25	0.5	0.5	0.5	0.25	0.25	0.5	0.5
Green cath	1.25	0.5	1	1	0.25	0.5	0.5	0.5	0.25	0.25	0.25	0.5
Urine	1	1	1	0.25	0.25	0.5	1	1	1	0.5	0.25	0.5
GV	1	1	1	0.25	0.25	0.5	0.5	0.25	0.25	0.25	0.25	0.5
Abscess	1	1	1	0.25	0.25	0.5	1	1	1	0.25	0.25	0.25
Throat swab	0.25	0.25	0.5	1	1	0.25	1	1	1	0.5	0.5	0.25
Aspirates	0.25	0.5	0.5	0.5	0.25	0.5	0.5	0.5	0.25	0.25	0.5	0.5
Wound	1	1	1	0.25	0.25	0.5	0.25	0.5	0.5	0.5	0.25	0.5
Nasal swab	0.25	0.25	0.5	1	1	1	1	1	0.25	0.25	0.25	0.25
Surgical	0.25	0.5	0.5	0.5	0.25	0.25	0.5	0.5	0.5	0.25	0.5	0.25
ATCC 27853	0.25	0.5	0.5	0.5	0.25	0.25	0.5	0.5	0.25	0.25	0.25	0.25
Mean MIC*	0.75	0.625	0.75	0.625	0.625	0.625	0.625	0.625	0.625	0.375	0.375	0.375

*For all samples. Cath, catheter; GV, greenish ventilator; MIC, minimum inhibitory concentrations (µg/mL); Surgical, sourced during surgery.

of *P. aeruginosa* were 23 and 2 mm, respectively, compared with 29 and 20 mm, respectively, for organic ethanoic acids. Among the 10 clinical isolates, the strain from the wound sample was resistant to 8 of the 12 standard synthetic chemical antibiotics, and sensitive to piperacillin/ tazobactam (PTC) and tetracycline (TC) with a zone diameter of 20 mm. This strain also had intermediate sensitivity toward gentamicin (GM) and meropenem (MP) with zone diameters of 9 and 11 mm, respectively. The clinical *P. aeruginosa* isolates from the wound sample were the most multidrug-resistant among the strains isolated herein, with different degrees of sensitivity to all tested antimicrobial agents. These results showed that organic ethanoic acids were more effective than standard synthetic antibiotics even against the most multidrug-resistant of the clinical *P. aeruginosa* isolates obtained herein.

Tables 1 and 3 show detailed comparison of the antimicrobial susceptibilities of all *P. aeruginosa* isolates from patients with nosocomial infections to standard synthetic chemical antibiotics and organic ethanoic acids. The mean MIC of standard antibiotic dilutions in e-tests and of organic ethanoic acids ranged from 0.375 – 0.750 µL/mL and 0.25 – 0.875 µL/mL, respectively for all *P. aeruginosa* isolates tested. Tables 2 and 4 respectively show details of the MIC of the standard synthetic chemical antibiotics and organic ethanoic acids for all *Pseudomonas aeruginosa* isolates from patients with nosocomial infections. The mean values of the MBC of organic ethanoic acid ranged from 0.5 – 1.25 µL/mL toward all

Table 3. Comparison of diffusion zone diameters (mm) among *Pseudomonas aeruginosa* isolates that were susceptible to organic ethanoic acids in conventional assays.

Sample	Organic ethanoic acid sources				
	Apple cider	Sugar molasses	Dates	Grapes	Grains
Green pus	22	22	22	22	22
Green catheter	20	22	19	22	22
Urine	21	21	20	22	24
Greenish ventilator	22	22	23	22	23
Abscess	27	22	24	22	24
Throat swab	24	22	23	26	26
Aspirates	23	26	24	22	22
Wound	24	22	22	22	22
Nasal swab	22	22	22	22	22
Surgical samples	25	22	22	22	22
ATCC 27853 (Control)	29	26	26	21	20
Mean Zone diameter (mm)	24.5	24	21.5	23.5	23

Table 4. Comparison of minimum inhibitory concentrations of organic ethanoic acids among *Pseudomonas aeruginosa* isolates.

Sample	Organic ethanoic acids (µg/mL)				
	Apple cider	Sugar molasses	Dates	Grapes	Grains
Green pus	0.25	0.5	1	1	1
Green catheter	0.25	0.25	1	1	0.75
Urine	0.25	0.5	1	0.75	1
Greenish ventilator	0.25	0.25	1	0.5	1
Abscess	0.25	0.5	0.75	0.5	0.75
Throat swabs	0.25	0.25	0.5	0.5	1
Aspirates	0.25	0.75	0.75	1	0.5
Wounds	0.25	0.75	0.75	0.75	0.5
Nasal swab	0.25	0.25	1	0.5	1
Surgical sources	0.25	0.75	1.25	1	0.5
ATCC 27853	0.25	0.25	0.5	0.5	0.5
Mean MIC (µg/mL)*	0.25	0.5	0.875	0.75	0.75

MIC, minimum inhibitory concentrations. *Derived from all samples.

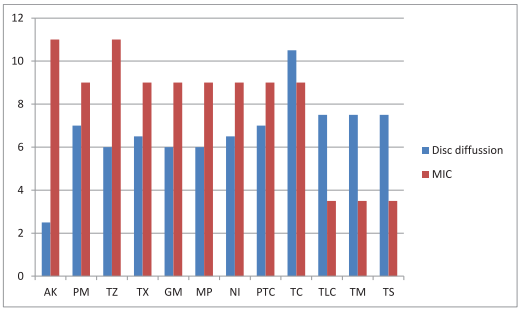

Fig. 1. Comparison of mean e-test susceptibility of 10 clinical *Pseudomonas aeruginosa* isolates to standard synthetic chemical antibiotics

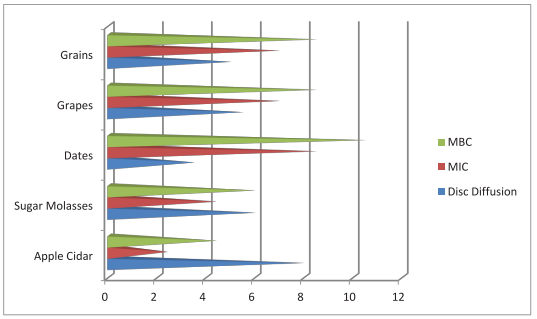

Fig. 2. Comparison of mean conventional susceptibility of 10 clinical *Pseudomonas aeruginosa* isolates to organic ethanoic acids derived from different sources.

Table 5. Comparison of minimum inhibitory concentrations of organic ethanoic acids among *Pseudomonas aeruginosa* isolates determined by conventional assays.

	Organic ethanoic acids (µg/mL)				
Sample	Apple cider	Sugar molasses	Dates	Grapes	Grains
Green pus	0.5	0.75	1.25	1.25	1.25
Green catheter	0.5	0.5	1.25	1.25	1
Urine	0.5	0.75	1.25	1	1.25
Greenish ventilator	0.5	0.5	1.25	0.75	1.25
Abscess	0.5	0.75	1	0.75	1
Throat swabs	0.5	0.5	0.75	0.75	1.25
Aspirates	0.5	0.5	1	1.25	0.75
Wound	0.5	0.5	1	1	0.75
Nasal swabs	0.5	0.5	1.25	0.75	1.25
Surgical	0.5	0.1	1.5	1.25	0.75
ATCC 27853	0.5	0.5	1	0.75	0.75
Mean MIC (µg/mL)	0.5	0.75	1.25	1	1

*For all samples. MIC, minimum inhibitory concentrations.

P. aeruginosa isolates. Table 5 shows a detailed comparison of the MBC of organic ethanoic acids for all the *P. aeruginosa* isolates.

The comparative findings of the antimicrobial susceptibility test values for the 10 clinical isolates of *P. aeruginosa* derived from patients with nosocomial infections suggests that organic ethanoic acid is significantly more effective than standard synthetic antibiotics.

CONCLUSIONS

The most important finding of the study was that the ATCC 27853 standard strain of *P. aeruginosa* was more susceptible to organic ethanoic acids than to standard synthetic antibiotics.

The susceptibility of the 10 clinical isolates of *P. aeruginosa* derived from the patients was variable compared with that to organic ethanoic acids. This finding indicates that the multidrug-resistant strains in the clinical isolates might be the result of excessive antibiotic administration, especially during prolonged hospitalization. Multidrug-resistant strains emerge due to constant disregard for warnings about antibiotic use published by the World Health Organization (WHO). The present findings showed that natural remedies such as organic ethanoic acids can serve as an alternative to synthetic antibiotics not only for eradicating multidrug-resistant organisms but also for other infections, which together with zero side effects could help save many lives.

ABBREVIATIONS

AK, amikacin; ATCC 27853- Standard *Pseudomonas aeruginosa* strain; GM, gentamicin; I, intermediate; MP, meropenem; NI, nitrofurantoin; PM, cefepime; PTC, piperacillin/ tazobactam; R, resistant; S, sensitive; SZD, susceptibility zone diameter (mm); TC, tetracycline; TLC, ticarcillin/ clavulanic acid; TM, tobramycin; TS, trimethoprim; TX, ceftriaxone; TZ, ceftazidime.

ACKNOWLEDGEMENTS

We acknowledge Ibn Sina National College, Jeddah (Kingdom of Saudi Arabia) Administration for their constant support and encouragement due to which this project came to existence.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript and/or the Supplementary Files.

ETHICS STATEMENT

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

REFERENCES

1. Alaa S, Muazzam SM, Mohannad S. Role of *Pseudomonas aeruginosa* in nosocomial infection. *Int J Health Sci*. 2018; **6**(1):211–217.
2. Yetkin G, Otlu B, Cicek A, Kuzucu C, Durmaz R. Clinical, microbiologic, and epidemiologic characteristics of *Pseudomonas aeruginosa* infections in a University Hospital, Malatya, Turkey. *Am J Infect Control*. 2006;**34**(4):188–92. https://doi.org/10.1016/j.ajic.2005.11.010
3. Torabi S, Falak-ul-Aflaki B, Moezzi F. In vitro antimicrobial drug-resistance of urinary tract pathogens in patients admitted to Vali-e-Asr hospital wards. *ZUMS J*. 2007;**15**(61):79–88.
4. Lida B, Norkhoda S, Farid Azizi J, Hassan H, Sattar MD, Ali H, Sobhan G, Iraj P. E-test antibiotic susceptibility of *E. coli* strains isolated from hospital acquired infections of Imam Khomeini hospital, Ilam, *Iran J Bas Res Med Sci.* 2017**;4**(1):17–21. https://doi.org/10.18869/acadpub.jbrms.4.1.17
5. Muazzam SM, Al Hasel HMB, Majid DH, Momen TN, Al Hazmi HAM, Al Jeddani FMS, Al Malaki RTW, Khan AA, Iqubal SMS. *Orient J Chem*. 2019**;35**(6):16951701.
6. Nagoba BS, Selkar SP, Wadher BJ, Gandhi RC. Acetic acid treatment of pseudomonal wound infections. *J Infect Public Heal***.** 2013;**6**:410–415. https://doi.org/10.1016/j.jiph.2013.05.005
7. Luber P, Bartelt E, Genschow E, Wagner J, Hahn H. Comparison of broth microdilution, E test, and agar dilution methods for antibiotic susceptibility testing of *Campylobacter jejuni* and *Campylobacter coli*. *J Clin Microbiol*. 2003;**41**(3):1062–1068. https://doi.org/10.1128/JCM.41.3.1062-1068.2003
8. Nagoba BS, Deshmukh SR, Wadher BJ, Patil SB. Acetic acid treatment of pseudomonal postoperative wound infection. *J Hosp Infect*. 1997;**36**:243–244. https://doi.org/10.1016/S0195-6701(97)90201-2
9. Al-Ibran E, Khan M. Efficacy of topical application of 1% acetic acid in eradicating pseudomonal infections in burn wounds. *J Dow Univ Health Sci*. 2010;**4**:90–93.
10. Bagewadi ZK, Muddapur UM, Madiwal SS, Mulla SI, Khan AA. Biochemical and enzyme inhibitory attributes of methanolic leaf extract of *Datura inoxia* Mill. *Environ Sustain*. 2019;**2**:75. https://doi.org/10.1007/s42398-019-00052-6
11. Gouse BS, Muazzam SM, Gokul SS, Ranjith MS. The efficacy *of Cinnamomum tamala* as a potential antimicrobial substance against the multi-drug resistant enterococcus faecalis from clinical isolates. *Int J Pharm Pharmaceutical Sc*. 2017;**9**(10):267.
12. Bisht CMS, Iqubal SMS, Khan AA, Tasneem M, Dawoud A, Gamal M, Singh SK, Asghar BH. Antifungal activity of *Salvii Jordanii. J Pure Appl Microbiol*. 2019; **13**(3):1611. https://doi.org/10.22207/JPAM.13.3.34