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Article

Effect of some antiseptics on elastase production from *Pseudomonas aeruginosa*.

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ABSTRACT

The present study was carried out to investigate the effect of clinically used antiseptics on elastase production from *Pseudomonas aeruginosa*. 39 clinical isolates were collected from wounds 10 (25.64%) and burns 29 (74.35%) from hospitalized patients in Baghdad city. Elastin preparation by the autoclaving method yielded 10.5 gm of elastin powder from (250 gm) of sheep's lungs, 3.6 gm from (50 gm) of sheep's bladder, and sheep's ligamentum nuchae (65 gm) yielded 15 gm. All *P. aeruginosa* isolates were tested for their ability to produce elastase by being cultivated on elastin nutrient agar and observed for the enzyme's activity. The bacteria that make elastase grew, and a clear border emerged surrounding the growth after 24 hours. 32 (82.05%) of *P. aeruginosa* isolates produced the enzyme on the elastin nutrient agar. Elastase-producing *P. aeruginosa* was tested quantitatively using the ELISA reader and spectrophotometer at (A_{495}) to detect the released amount of Congo red dye from the degradation of the elastin Congo red. *P. aeruginosa* (P41) showed the highest elastolytic activity; thus, it was selected to determine the effect of the sub-MIC of the antiseptics on elastase production. The results showed that acetic acid was the best agent to inhibit elastase production, followed by silver nitrate, hydrogen peroxide and ethanol in descending order.

Keywords: Elastase; Elastin; Pseudolysin; Antiseptics; Acetic acid; *Pseudomonas aeroginosa*

INTRODUCTION

Compromises in the skin, whether accidental or intentional, allow bacterial pathogens to enter the body, causing skin and soft tissue infection (SSTI). SSTI is a very common infectious illness, with an estimated 14.2 million ambulatory care attendances in the US attributable to it.[1] Increasingly, topical antibiotics and antiseptics are used to prevent skin infections, especially in individuals colonized with bacterial pathogens.[2]

P. aeruginosa is a Gram-negative, aerobic, rod-shaped bacterium that belongs to the Pseudomonadaceae bacterial family.[3] Approximately 10% to 20% of hospital infections are caused by this pathogen, which can form biofilm.[4] Clinically and epidemiologically, *P. aeruginosa* is important. It causes nosocomial infections from non-fermenting Gram-negative bacilli and opportunistic infections in immunocompromised patients.[5] *P. aeruginosa's* virulence factors are cell-associated and extracellular. Regulatory circuits and signaling systems control these virulence

factors.[6] Elastase, called LasB or pseudolysin, is a zinc-dependent thermolysin metalloprotease. LasB encodes it. It's the most common extracellular protease.[7] *P. aeruginosa* produces elastase in order to degrade the host's elastin.[8] It also cleaves surfactant proteins, cytokines, immunoglobulins, and inflammasome components, interfering with bacterial clearance.[9]

Elastin is produced by lysyl oxidase by crosslinking tropoelastin. Tropoelastin is a 60-70 kDa fibroblast precursor protein.[10] Elastin gives tissues resilience and elasticity. Aorta (57%) and elastic ligaments (50%), as well as major arteries (32%), lungs (7%) and skin (5%).[11]

Pseudolysin mechanism of action. When pseudolysin molecules contact elastin fibers, they attach to the fibers' hydrophobic regions. After binding, pseudolysin attacks weak bonds between hydrophobic amino acid residues in elastin's hydrophobic regions, forming grooves and holes on the fiber's surface. Over time, grooves and holes grow on the elastin fiber's exterior until it shatters into fragments, which are then broken down into free amino acids and smaller peptides.[12]

Pathogen-secreted virulence proteins can potentially impair various physiological processes in the host, including wound healing.[13] During the host-pathogen interaction, pseudolysin targets elastin, collagen III and collagen IV from the interstitial extracellular matrix, degrades immunoglobulin A and G, and suppresses fibroblast development. [13] Pseudolysin inhibits plasminogen-to-plasmin conversion, causing wound fibrin clots.[14]

It has become common practice to equate proper wound care with the topical prevention and treatment of microbial infection.[15] In essence, good wound treatment necessitates the removal of contaminants while causing little tissue damage.[16] Antiseptics have a variety of applications and indications, including their use as prophylactic anti-infective agents for open wounds, such as lacerations, abrasions, burns, and chronic ulcers, which has been a source of heated debate for several years.[17] Numerous writers advise avoiding using them on open wounds due to cytotoxicity. Some are too harsh for wounded skin because they include detergents.[17]

There is a lot more information that has to be gathered on the efficacy of the many techniques that are presently employed for the topical treatment of open wounds, whether they are acute or chronic.[18] Biguanides (chlorhexidine), alcohols (ethyl alcohol), chlorinated compounds, iodinated compounds, silver-based compounds, peroxygens and quaternary ammonium compounds are only a few antiseptic groups. Povidone-iodine, chlorhexidine, alcohol, acetate, hydrogen peroxide (H_2O_2), silver nitrate, and sodium hypochlorite are the most regularly utilized items in clinical practice today.[17] Antiseptics are toxic to different components of bacterial cell metabolism elements instead of specific sites of antibiotic activity like enzyme inhibition and are largely harmless to tissues.[19]

MATERIALS AND METHODS

Sample collection and identification of bacterial isolates

39 bacterial isolates from clinical sources of wounds and burns of hospitalized patients were collected from Baghdad city. Identification of *P. aeruginosa* by morphological examination of their colonies, including the colony shape, texture, color, odor, and edges of the growth on MacConkey Agar and Cetrimide pseudo agar were examined as primary diagnostic tests incubated at 37°C and for (24 hrs.). A touch of isolated single colony was fixed and dyed with the Gram stain on a microscopic slide for microscopic examination. The color of the Gram reaction and the structure and arrangement of the cells were all recorded.[20] Biochemical tests were done on the *P. aeruginosa* isolates, including oxidase, catalase, citrate, Indole, Methyl red, and Voges-Proskauer. [21,22]

Preparation of a medium to detect the ability of the bacterial isolates to produce elastase.

The cleaned lungs, bladder, and ligamentum nuchae of sheep were autoclaved in flasks with a gauze plug that does not fit tightly filled with 20 liters of distilled water in one atmosphere (for 45 min.). The autoclave stage is repetitive (4 times) and replacing the used water with distilled water till there is no evidence of protein in the flasks.[23] We used elastin powder from lungs, bladder and ligamentum nuchae with (2.5%) nutrition agar to make elastin agar medium. It was then put through an autoclave sterilization process at a temperature of 121° and a pressure of 15 bar/in^2 for fifteen minutes. A plastic petri plate was used to allow the agar solution to harden.[24] Elastin broth medium was prepared by adding 1% elastin powder from the Extraction of insoluble elastin mentioned above from any of the three sources (lungs, bladder and ligamentum nuchae) to 2.5% nutrient broth suspended in (100 ml) of distilled water and poured into autoclavable tubes, sterilized under moist heat by autoclave at (121°C, 15 bar/in^2 pressure and 15 minutes). Finally, the tubes were kept cold (4°C) pending usage.

The powdered elastin from sheep ligamentum nuchae was dissolved in 100 ml of saturated aqueous Congo Red solution and gently stirred for (3 hrs.). In order to remove excess dye, distilled water and acetone were used to repeatedly wash the Buchner funnel many times. Washing proceeded until no more color was removed. After washing with ether and subjecting the colored mass to 45°C temperature, the colored mass was eventually dried.[25]

Detection of elastase production

Qualitative assay

The streaking method is used to determine elastase-producing bacteria in an elastin agar medium containing 1% elastin and 2.5% nutrient agar. Each isolate was grown separately on three different elastin sources at 37°C for (24 hrs.).[26]

Quantitative assay

Each *P. aeruginosa* isolate was cultured for 24 hours at 37°C in a 200 rpm shaking incubator in nutritional broth with 0.2% elastin (ligamentum nuchae) media. Centrifugation at 5000 g for 10 minutes removed the cells, which were subsequently sterilized using a Millipore filter (0.22m). One ml of sterilized supernatant was incubated for 30 minutes at 200 rpm in a shaking incubator at room temperature with 20 µg elastin Congo red (ECR). The process was stopped by adding 0.1 M NaOH. As a final step, soluble Congo red dye was extracted from the supernatant by centrifugation for 10 minutes at room temperature with 10000 g of centrifugal force. Soluble Congo red dye released into the supernatant was measured at A_{495}. As a baseline, we utilized fresh NB medium.[27]

Antibiotic susceptibility test according to Vitek 2 system

As the manufacturer (bioMérieux/France) instructed, the testing was conducted. Ten antibiotics for the *P. aeruginosa* isolates including, Cefazolin, Piperacillin/Tazobactam, Ceftazidime, Cefepime, Meropenem, Amikacin, Gentamicin, Ciprofloxacin, Tigecycline and Colistin were used.

Determination of the Minimum Inhibitory Concentration (MIC) for antiseptics on growth of elastase-producing isolates

Sterilized microtiter plates of 96 wells were prepared and used. Muller Hinton broth was used to serially dilute 180 µL of different concentrations of test material (antiseptics) which includes chlorhexidine, povidone iodine, H_2O_2 and Acetic Acid with concentration (0.5-0.004) % separately, while alcohol (40-5%) and

Silver nitrate (0.032-0.001%). 20 µL of suspension of the bacteria (1.5×10^8 CFU/ml) was added to each row except the negative control. There was a set of controls on each plate: Positive control row with the Muller Hinton broth (180 µL) and bacterial inoculation (20 µL) except for the test substance; Negative control row with 200 µL of Muller Hinton broth, except for the bacteria and the material being tested. The triplicate plates were incubated at 37° C for 24 hours (24 hrs.). To each well, 10 µL of resazurin reagent solution was added following the incubation. The color shift of the resazurin reagent was evaluated. Positive results were noted when the color changed from purple to pink or remained purple, indicating a negative result. The MIC value is the smallest concentration of the test material at which color change occurred, and it was determined using the average concentrations of three readings.[28]

Effect of antiseptics on elastase production from P. aeruginosa.

Effect of sub-MIC of antiseptics on elastase production from the highest elastase producer, *P. aeruginosa* isolates done as according to Jian and colleagues,[27] with modification. The highest elastase producer, *P. aeruginosa* isolate, was cultured in a media containing nutrient broth and 0.2% elastin (ligamentum nuchae) and then adjusted to the concentration of sub-MIC of each antiseptic and incubated for 24 hours at 37°C in the shaker incubator 200 rpm. Centrifugation was used to eliminate the bacterial cells at 5000 g for (10min) and then sterilized with a paper filter (0.22µm). One milliliter of sterile supernatant was incubated with (20 µg) of elastin Congo red (ECR) for (30 min.) at a shaking incubator at 200 rpm at room temperature. To stop the reaction, 0.1 M NaOH was added. Then, insoluble ECR was removed by centrifugation at 10000 g for 10 min at room temperature, and soluble Congo red dye released into the supernatant was measured at A_{495}. Fresh NB medium was used as the blank control.

RESULTS

Identification of bacterial samples

On MacConkey agar, the *P. aeruginosa* colonies appeared as pale yellow, non-fermenter smooth spherical colonies and gave grape-like or tortilla-like smell. *P. aeruginosa* colonies after 24 hours of incubation on Blood agar media, most bacterial isolates developed β-hemolysis and developed as huge flat bacterial colonies with a grape-like odor.[29] Pseud Cetrimide agar was used for the identification of *P. aeruginosa* in which Cetrimide increases the synthesis of two pigments, blue-green pigment as pyocyanin and fluorescent pigment as pyoverdine, and works to inhibit the majority of other bacteria.[30] Gram stain for *P. aeruginosa* showed that gram-negative (red) rods seem like a single, short chain or groups, which they showed under a compound light microscope at 40X and 100X.[31] Table 1 explains the results of the biochemical examination.

Bacteria	**Biochemical Tests**			
	Gram stain	Oxidase	Catalase	Urease
P. aeruginosa	(-)	(+)	(+)	(-)
	IMViC tests			
	Indole	Methyl red	Voges-Proskauer	Citrate utilization
P. aeruginosa	(-)	(-)	(-)	(+)

Table 1. The microscopic and biochemical test results of bacterial isolates.

The *P. aeruginosa* isolates with the greatest elastase production could be definitively identified using the Vitek 2 system, as shown in Appendix A. The distribution of *P. aeruginosa* isolates according to the source of the specimen is Burns 29 (74.35%) and wounds 10 (25.64%). Ten types of antibiotics were tested by Vitek 2 system for *P. aeruginosa*. They demonstrated several forms of resistance against various antibiotics, such as the following: Cefazolin (100%), Tigecycline (88%), Meropenem, Amikacin, Gentamicin and Ciprofloxacin all at (50%), Cefepime (38%), Piperacillin/Tazobactam and Ceftazidime at (25%) and Colistin (0%) as shown in Appendix A.

Elastin preparation

The elastin preparation method [23] yielded 10.5 gm of elastin powder from (250 gm) of sheep's lungs, 3.6 gm of elastin powder from (50 gm) of sheep's bladder and elastin powder from sheep's ligamentum, nuchae (65 gm) yielded 15 gm of elastin powder. Noted that the sheep's ligamentum nuchae yielded the highest amount of the final product.

Screening of elastase production from bacterial isolates

Qualitative assay

According to our findings, many clinical *P. aeruginosa* isolates were able to break down elastin due to carrying the lasB gene,[32], an important virulence factor that helps *P. aeruginosa* break down elastin protein. The *P. aeruginosa* isolates that make elastase were formed, and a clear border emerged surrounding the growth in (24 hrs.) as shown in Figure 1. From 39 *P. aeruginosa* isolates, 32 (82.05%) produced elastase, and 7 isolates (17.94%) didn't.

Figure 1. Elastase producing *P. aeruginosa*.

Quantitative assay

The results (Figure 2) showed that the isolate of *P. aeruginosa* (P41) has the highest elastolytic activity among other isolates in both the ELISA reader and the spectrophotometer readings. Thus, this isolate, *P. aeruginosa* (P41), was qualified and used to detect the anti-elastase activity of antiseptics.

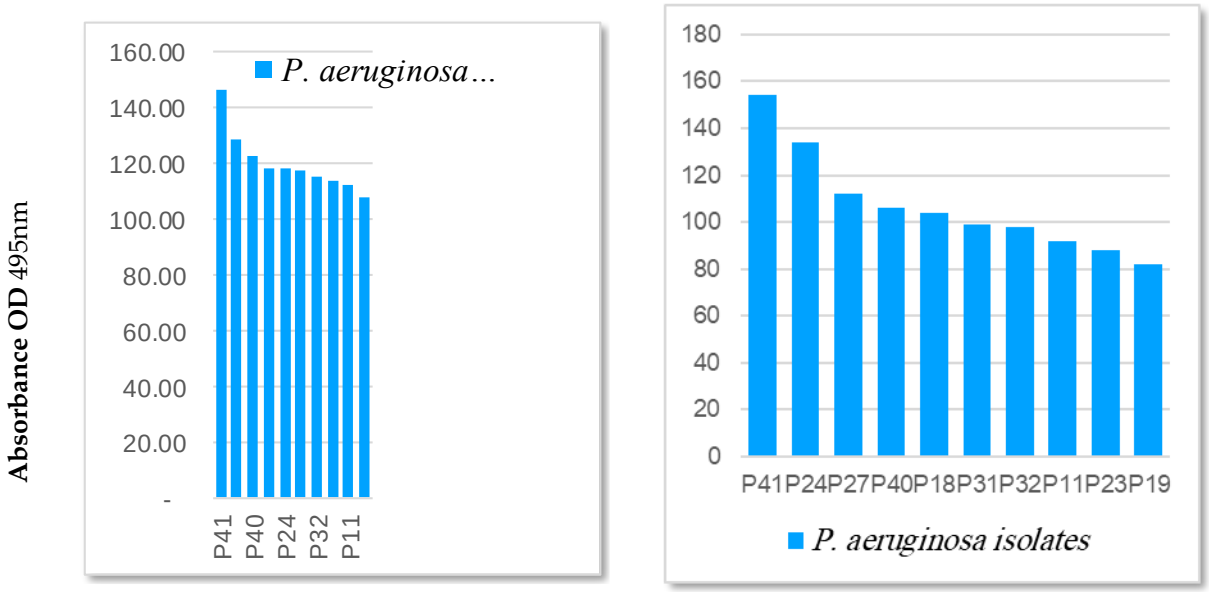


Figure 2. ELISA (A) and Spectrophotometer reader (B) readings at (A_{495}).

Minimum Inhibitory Concentration (MIC) for antiseptics

Determination of the Minimum Inhibitory Concentration (MIC) for antiseptics on the growth of elastase-producing isolate was measured according to the Resazurin reagent measurement method. Results show that antiseptics could inhibit the growth of *P. aeruginosa* (P41) in the concentrations shown in Table 2.

Antiseptics	MIC	Sub-MIC
Povidone Iodine	0.016%	0.008%
Alcohol	10%	5%
Chlorhexidine	0.032%	0.016%
H_2O_2	0.25%	0.125%
Acetic acid	0.125%	0.064%
Silver Nitrate	0.004%	0.002%

Table 2. Minimum Inhibitory Concentration (MIC) for antiseptics on the growth of elastase-producing *P. aeruginosa* (P41)

Effect of antiseptics on elastase production of P. aeruginosa.

After the determination of the sub-MIC of the antiseptics, an assay was done to determine the effect of antiseptics on the elastase production of *P. aeruginosa* (P41). Soluble Congo red dye released into the supernatant was measured at A_{495} on the ELISA reader and the spectrophotometer. The results are shown in Figure 3. The results show the superiority of vinegar (acetic acid) in inhibiting the elastase enzyme or even interfering in its regulation, followed by hydrogen peroxide and silver nitrate as effective elastase inhibitors. The results also show the low effect of antiseptics such as povidone-iodine, alcohol and chlorhexidine on elastase activity compared to acetic acid.

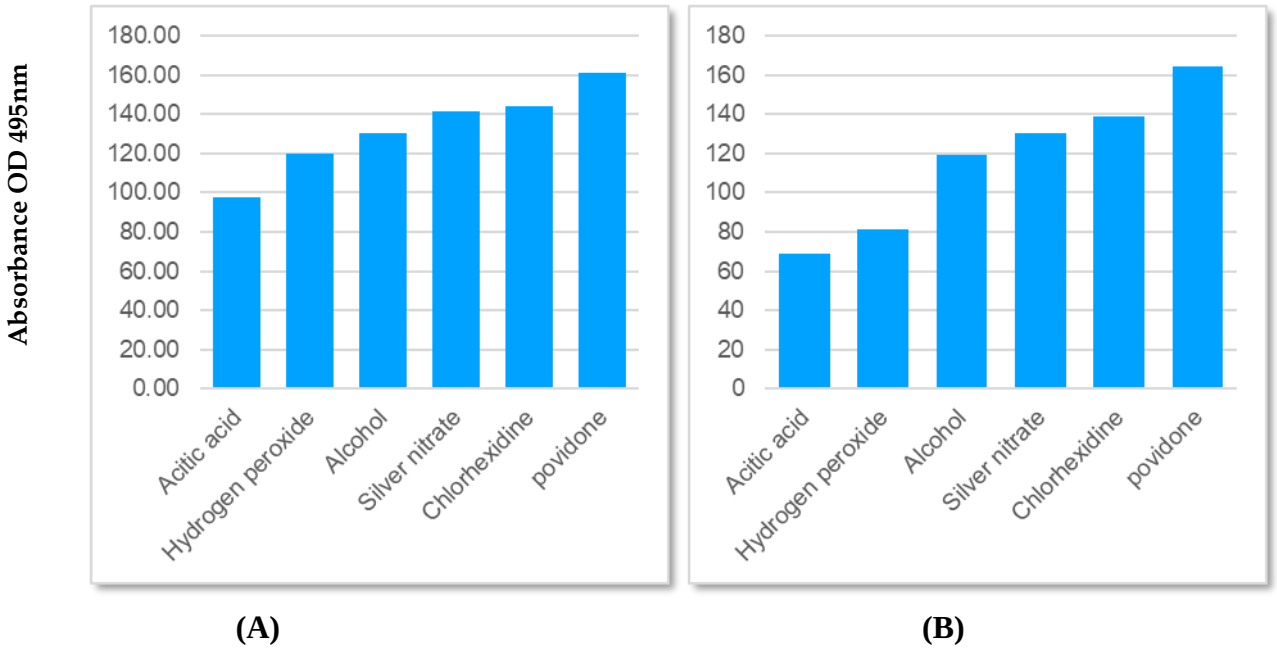


Figure 3. Effect of antiseptics on elastase produced from *P. aeruginosa* measured in ELISA reader (A) and Spectrophtometer reader (B)

DISCUSSION

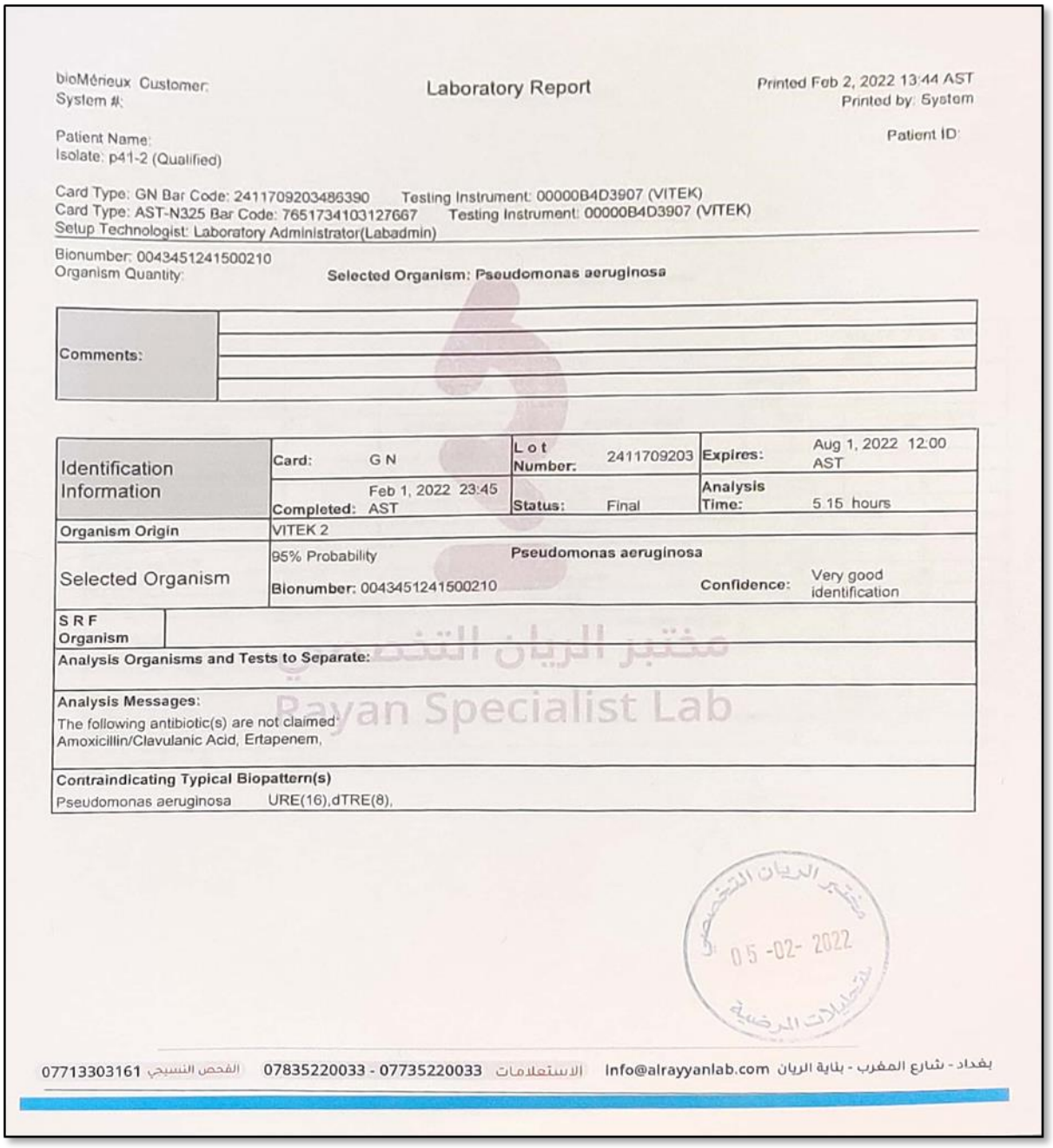
Temperature and pH affect how an enzyme works. Each enzyme has an ideal temperature and pH at which it works best. The effect of acidic H+ ions or basic OH- ions on an enzyme's activity is probably caused by a change in the shape of the active sites or the area around them.[33] The effect of the acidic environment on purified elastase activity was clarified by Shinyar and colleagues,[34] that the optimum pH is (7.0) and the acidic pH of (4.0) decreases the elastase activity. On the other hand, Wei and colleagues,[35] found that pseudolysin was active in the gel at pH (2.0) when they investigated its properties as an aciduric gluten-degrading enzyme with high therapeutic potential for celiac disease.

Historically, Phillips and colleagues 36 were the first to report the use of acetic acid as a topical agent for treating superficial wounds infected by *P. aeruginosa*. Alexandre and colleagues [37] screened the sensitivity of *P. aeruginosa* (PAO1) toward acetic acid produced by Lactobacillus spp. that inhibits the growth of many bacteria through their undissociated forms at low pH. Since Lactobacillus spp. produce acetic acids; five isolates reduced the activity of elastase after 22 hours of co-incubation with *P. aeruginosa* (PAO1) at 37 °C in BHI broth

CONCLUSIONS

Burn and wounds are especially susceptible to infection from gram-negative bacteria, with almost half of the bacterial isolates diagnosed as *Pseudomonas aeruginosa*. The ovine's ligamentum nuchae yielded the highest amount of elastin. *P. aeruginosa* is the highest elastase-producing bacteria. All elastase-producing *P.* aeruginosa were susceptible to Colistin, while the same isolates were resistant to Cefazolin, and most were resistant to Tigecycline. The sub-MIC of antiseptics shows some inhibitory effect on elastase; acetic acid was the best inhibitor for the enzyme, while povidone-iodine was the least inhibitory antiseptic.

Appendix A



bioMérieux Customer:
System #:

Laboratory Report

Printed Feb 2, 2022 13:44 AST
Printed by: System

Patient Name:
Isolate: p41-2 (Qualified)

Patient ID:

Card Type: GN Bar Code: 2411709203486390 Testing Instrument: 00000B4D3907 (VITEK)
Card Type: AST-N325 Bar Code: 7651734103127667 Testing Instrument: 00000B4D3907 (VITEK)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 0043451241500210
Organism Quantity:

Selected Organism: Pseudomonas aeruginosa

Comments:	

Identification Information	Card:	G N	Lot Number: 2411709203	Expires:	Aug 1, 2022 12:00 AST
	Completed:	Feb 1, 2022 23:45 AST	Status: Final	Analysis Time:	5.15 hours
Organism Origin	VITEK 2				
Selected Organism	95% Probability		Pseudomonas aeruginosa		
	Bionumber: 0043451241500210			Confidence:	Very good identification
SRF Organism					

Analysis Organisms and Tests to Separate:

Analysis Messages:
The following antibiotic(s) are not claimed:
Amoxicillin/Clavulanic Acid, Ertapenem,

Contraindicating Typical Biopattern(s)
Pseudomonas aeruginosa URE(16),dTRE(8),

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References

1. Hersh, A. L.; Chambers, H. F.; Maselli, J. H.; & Gonzales, R. National trends in ambulatory visits and antibiotic prescribing for skin and soft-tissue infections. *Archives of internal medicine,* **2008**; *168(14),* 1585-1591.
2. Septimus, E. J.; & Schweizer, M. L. Decolonization in prevention of health care-associated infections. *Clinical microbiology reviews,* **2016**; *29(2),* 201-222.
3. Todar, K. Todar's online textbook of bacteriology. University of Wisconsin-Madison Department of Bacteriology. **2005**.
4. Fazeli, H.; Akbari, R.; Moghim, S.; Narimani, T.; Arabestani, M. R.; & Ghoddousi, A. R. Pseudomonas aeruginosa infections in patients, hospital means, and personnel's specimens. Journal of research in medical sciences: *the official journal of Isfahan University of Medical Sciences,* **2012**; *17(4),* 332.

5. Kollef, M. H. Antibiotics for the critically ill: more than just selecting appropriate initial therapy. *Critical Care*, **2013**; *17(3)*, 1-3.
6. Balasubramanian, D.; Schneper, L.; Kumari, H.; & Mathee, K. A dynamic and intricate regulatory network determines Pseudomonas aeruginosa virulence. *Nucleic acids research*, **2013**; *41(1)*, 1-20.
7. Galdino, A. C. M.; Branquinha, M. H.; Santos, A. L.; & Viganor, L. Pseudomonas aeruginosa and its arsenal of proteases: weapons to battle the host. Pathophysiological Aspects of proteases, 381-397. **2017**.
8. Strateva, T.; & Mitov, I. Contribution of an arsenal of virulence factors to pathogenesis of Pseudomonas aeruginosa infections. *Annals of microbiology*, **2011**; *61(4)*, 717-732.
9. Yang, J.; Lee, K. M.; Park, S.; Cho, Y.; Lee, E.; Park, J. H.; ... & Yu, J. W. Bacterial secretant from Pseudomonas aeruginosa dampens inflammasome activation in a quorum sensing-dependent manner. *Frontiers in immunology*, **2017**; *8*, 333.
10. Kozel, B. A.; Rongish, B. J.; Czirok, A.; Zach, J.; Little, C. D.; Davis, E. C.; ... & Mecham, R. P. Elastic fiber formation: a dynamic view of extracellular matrix assembly using timer reporters. *Journal of cellular physiology*, **2006**; *207(1)*, 87-96.
11. Wise, S. G.; & Weiss, A. S. Tropoelastin. *The international journal of biochemistry & cell biology*, **2009**; *41(3)*, 494-497.
12. Yang, J.; Zhao, H. L.; Ran, L. Y.; Li, C. Y.; Zhang, X. Y.; Su, H. N.; ... & Zhang, Y. Z. Mechanistic insights into elastin degradation by pseudolysin, the major virulence factor of the opportunistic pathogen Pseudomonas aeruginosa. *Scientific reports*, **2015**; *5(1)*, 1-7.
13. Schmidtchen, A.; Holst, E.; Tapper, H.; & Björck, L. Elastase-producing Pseudomonas aeruginosa degrade plasma proteins and extracellular products of human skin and fibroblasts, and inhibit fibroblast growth. *Microbial pathogenesis*, **2003**; *34(1)*, 47-55.
14. Leduc, D.; Beaufort, N.; de Bentzmann, S.; Rousselle, J. C.; Namane, A.; Chignard, M.; & Pidard, D. The *Pseudomonas aeruginosa* LasB metalloproteinase regulates the human urokinase-type plasminogen activator receptor through domain-specific endoproteolysis. *Infection and immunity*, **2007**; *75(8)*, 3848-3858.
15. Wilson, J. R.; Mills, J. G.; Prather, I. D.; & Dimitrijevich, S. D. A toxicity index of skin and wound cleansers used on in vitro fibroblasts and keratinocytes. *Advances in skin & wound care*, **2005**; *18(7)*, 373-378.
16. Bianchi, J. The cleansing of superficial traumatic wounds. *British Journal of Nursing*, **2000**; *9(Sup3)*, S28-S38.
17. Drosou, A.; Falabella, A.; & Kirsner, R. S. Antiseptics on wounds: an area of controversy. *Wounds*, **2003**; *15(5)*, 149-166.
18. Edlich, R. F.; Rodeheaver, G. T.; Thacker, J. G.; Lin, K. Y.; Drake, D. B.; Mason, S. S.; ... & Vissers, R. J. Revolutionary advances in the management of traumatic wounds in the emergency department during the last 40 years: part I. *The Journal of emergency medicine*, **2010**;*38(1)*, 40-50.
19. Leaper, D. J.; & Durani, P. Topical antimicrobial therapy of chronic wounds healing by secondary intention using iodine products. *International Wound Journal*, **2008**; *5(2)*, 361-368.
20. Brooks, G. F.; Carroll, K. C.; Butel, J. S.; Morse, S. A.; & Mietzner, T. A. Microbiologia Médica de Jawetz, Melnick & Adelberg-26. AMGH Editora. **2014**.
21. Procop, G. W.; Church, D. L.; Hall, G. S.; & Janda, W. M. Koneman's color atlas and textbook of diagnostic microbiology. Jones & Bartlett Publishers. **2020**.
22. Goldman, E.; & Green, L. H. (Eds.). Practical handbook of microbiology. CRC press. **2015**.
23. Hall, D. A.; & Gardiner, J. E. The reaction between elastase and elastic tissue. 2. Preparation and properties of the enzyme. *The Biochemical journal*, **1955**; *59(3)*, 465–470. https://doi.org/10.1042/bj0590465
24. Jung, K. H.; & Kim, H. J. Development of an agar diffusion method to measure elastase inhibition activity using elastin-congo red. *Journal of microbiology and biotechnology*, **2006**; *16(8)*, 1320-1324.
25. Hall, D. A. The identification and estimation of elastase in serum and plasma. *Biochemical Journal*, **1966**; *101(1)*, 29.
26. Fujii, T.; Fukano, K.; Hirano, K.; Mimura, A.; Terauchi, M.; Etoh, S. I.; & Iida, A. A new serine protease family with elastase activity is produced by Streptomyces bacteria. *Microbiology*, **2020**; *166(3)*, 253-261.
27. Jian, L.; Mahnaz, R.; Stephanie, F.; Clare, C.; Jae, M.; Masanobu, S.; Alkis, P.; Peter, J, W; Sarah, V. Pseudomonas aeruginosa Exoprotein-Induced Barrier Disruption Correlates with Elastase Activity and Marks Chronic Rhinosinusitis Severity. Front. Cell. Infect. *Microbiol.* **2019**;*123*: 293–302.

28. Sarker, S. D.; Nahar, L.; & Kumarasamy, Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. *Methods*, **2007**; *42(4)*, 321-324.
29. Brooks, G.F.; Karen, C. and Carroll, M. D. Medical Microbiology. Jawetz, Melnick and adelberg's. 24th.ed. McGraw-Hill Companies, Inc. USA. **2007**; p:266-67.
30. Jabbar, H. S.; Mahdi, L. H.; & Auda, I. G. Antibacterial immunomodulatory and antibiofilm triple effect of Salivaricin LHM against P. aeruginosa urinary tract infection model. *International journal of biological macromolecules*, **2019**; *134*, 1132-1144.
31. Tille, P. Bailey & Scott's diagnostic microbiology-E-Book. Elsevier Health Sciences. **2015**.
32. Cathcart, G. R.; Quinn, D.; Greer, B.; Harriott, P.; Lynas, J. F.; Gilmore, B. F.; & Walker, B. Novel inhibitors of the Pseudomonas aeruginosa virulence factor LasB: a potential therapeutic approach for the attenuation of virulence mechanisms in pseudomonal infection. *Antimicrobial agents and chemotherapy*, **2011**; *55(6)*, 2670-2678.
33. Tijskens, L. M. M.; Greiner, R.; Biekman, E. S. A.; & Konietzny, U. Modeling the effect of temperature and pH on activity of enzymes: the case of phytases. *Biotechnology and bioengineering*, **2001**; *72(3)*, 323-330.
34. Shinyar, Y.A; Khalaf, K.J; Saleh, T.H. Effect of some chemical materials on elastase produced from *Pseudomonas aeruginosa*. Unpublished master thesis, Mustansiriyah University. **2021**.
35. Wei, G.; Tian, N.; Valery, A. C.; Zhong, Y.; Schuppan, D.; & Helmerhorst, E. J. Identification of pseudolysin (lasB) as an aciduric gluten-degrading enzyme with high therapeutic potential for celiac disease. *The American journal of gastroenterology*, **2015**; *110(6)*, 899.
36. Phillips, I.; Lobo, A. Z.; Fernandes, R.; & Gundara, N. S. Acetic acid in the treatment of superficial wounds infected by Pseudomonas aeruginosa. *The Lancet*, **1968**; *291(7532)*, 11-13.
37. Alexandre, Y.; Le Berre, R.; Barbier, G.; & Le Blay, G. Screening of *Lactobacillus spp*. for the prevention of *Pseudomonas aeruginosa* pulmonary infections. *BMC microbiology*, **2014**; *14(1)*, 1-10.

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