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A Sea Hare L-Amino Acid Oxidase to Fight Multiple Antibiotic-Resistant *Staphylococcus Aureus*

Abstract

Staphylococcus aureus is a pathogen notoriously known for its ability to resist antibiotics. Here, we reported the activity of the sea hare *Aplysia dactylomela* ink L-AAO protein, dactylomelin-P, against 100 clinical isolates of multiple antibiotic-resistance *S. aureus* with a prevalence of 30% methicillin-resistant (MRSA). Dactylomelin-P was able to inhibit the growth of all isolates with inhibition zones average size of 17.90 ± 2.58 mm. Among the eleven commercial antibiotics tested, only vancomycin, quinupristin/dalfopristin and linezolid exhibited similar efficiency. These findings highlight the potential of dactylomelin-P against *S. aureus* and MRSA as well as support further research with dactylomelin-P as an antibacterial drug candidate.

Keywords: Antibacterial Protein; Sea Hare; MRSA

Research Article

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Introduction

S. aureus is a serious public health problem associated with superficial wounds to life-threatening infections, with hospital outbreaks and resistant clones emerging frequently [1]. Since the 1960s, oxacillin (methicillin)-resistant *S. aureus* (MRSA) has established itself as one of the most common causes of nosocomial infections, whose dissemination has been of alarming concern [2]. Therefore, the interest in discovering novel antibacterial agents with alternative mechanisms of action or unexploited bacterial molecular targets [3]. Since the discovery of penicillin, more than 23,000 natural products have been characterized [4]. Most of them were small secondary metabolites, however bioactive proteins has emerged concomitantly with advances in new analytical techniques for the isolation and characterization of these biomolecules.

Dactylomelin-P is a 60 kDa monomeric protein purified from the purple ink of the sea hare *Aplysia dactylomela* that exhibits a wide range antibacterial activity [5]. Several sea hares can excrete a deep purple ink when threatened by a predator. Besides pigments of algal origin, the ink is a rich source of toxic peptides and bioactive proteins (Figure 1).

Dactylomelin-P possess a minimum inhibitory concentration (MIC) of 0.1 µg µL^{-1} against *S. aureus* ATCC 25923, with a mechanism of action based on its L-amino acid oxidase (L-AAO) activity against L-lysine and L-arginine [16]. This represents a remarkable and economic mechanism of action related with the release of hydrogen peroxide (H_2O_2) directly on the site of infection as well as other intermediate products generated non-enzymatically with additional bactericide action [17]. Considering the activity of dactylomelin-P against *S. aureus* ATCC 25923 and the increasing MRSA prevalence worldwide, here we examined its activity against 100 clinical isolates of multiple antibiotic-resistant *S. aureus* with a prevalence of 30% methicillin-resistant (MRSA).

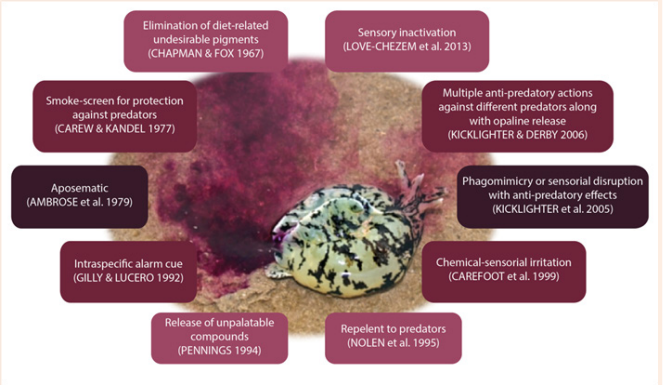

Figure 1: Chemical defense arsenal of the purple ink released by several sea hares [6-15].

Materials and Methods

Microorganisms and antibiotics

The microorganisms were kindly provided by the microbiology labs from three public hospitals of Fortaleza City, Ceará State,

Brazil: Albert Sabin Hospital; General Hospital of Fortaleza, Walter Cantídio Universitary Hospital, as well as from a private clinical laboratory. These isolates were obtained from abscesses, blood and urine cultures, cerebrospinal and pleural fluid, secretions, catheter tips and mitral valve. Eleven commercial antibiotics were tested against the isolates: oxacillin (1 µg/disk), penicillin (10 UI/disk); clindamycin (2 µg/disk), erythromycin (15 µg/disk), linezolid (30 µg/disk), sulfamethoxazole-trimethoprim (25 µg/disk), vancomycin (30 µg/disk), quinupristin/dalfopristin (15 µg/disk), rifampicin (5 µg/disk), norfloxacin (10 µg/disk) and chloramphenicol (30 µg/disk).

Antibiotic susceptibility test

Dactylomelin-P was prepared at a concentration of 100 µg mL^{-1} in 0.15 mol L^{-1} sterile saline. The susceptibility to antibiotics/dactylomelin-P was tested by the disk diffusion method on Mueller-Hinton agar as standardized by the Clinical and Laboratory Standards Institute (CLSI) [18].

Results and Discussion

Concerning the source of the isolates, the majority was from blood cultures and secretions (Figure 2). Obtaining 42% of the isolates in blood samples is alarming because *S. aureus* bacteremia is associated with high case fatality (20-30%) [19].

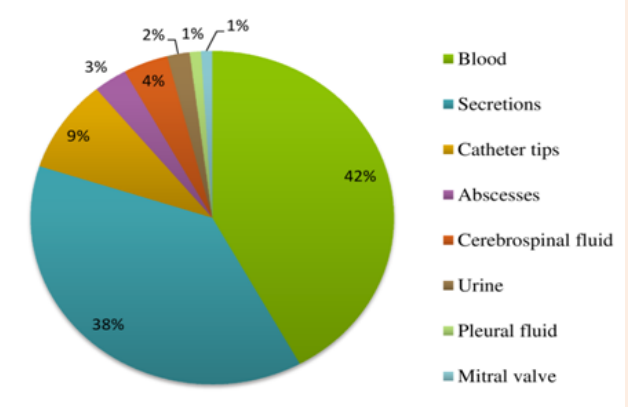

Figure 2: Sources of the clinical isolates of *S. aureus* tested in this current study.

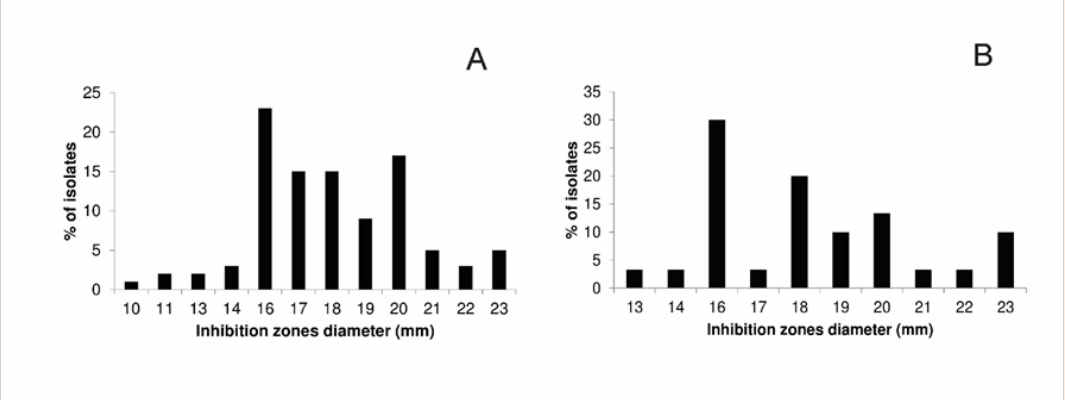

Figure 3: Inhibition zones diameters produced by dactylomelin-P against *S. aureus* clinical isolates **(A)** and MRSA strains **(B)**.

[a]Nd–not determined

Table 1: Percentage of *S. aureus* strains resistant to commercial antibiotics among 100 clinical isolates.

Source	Number of Strains	Resistant Strains (%)											
		OXA	PEN	CLI	ERY	LZD	SXT	VAN	QD	RIF	NOR	CHL	DACT
Wound	38	19	100	37	50	0	35	0	0	5	50	50	0
Catheter	9	55	100	80	33	0	55	0	0	22	50	50	0
Hemoculture	42	38	100	26	65	0	58	0	0	7	30	70	0
Urine culture	2	0	100	0	0	0	50	0	0	Nd[a]	Nd	Nd	0
Abscess	3	0	100	0	67	0	35	0	0	Nd	33	65	0
Liquor	5	20	100	0	40	0	60	0	0	35	20	0	0
Mitral valve	1	100	100	0	100	0	100	0	0	Nd	0	0	0
Total of strains	100	30	100	28	58	0	43	0	0	11	18	11	0

OXA: Oxacillin; PEN: Penicillin; CLI: Clindamycin; ERY: Erythromycin; LZD: Linezolid; SXT: Sulfamethoxazole-trimethoprim; VAN: Vancomycin; QD: Quinupristin/dalfopristin; RIF: Rifampicin; NOR: Norfloxacin; CHL: Chloramphenicol; DACT: Dactylomelin-P

All the isolates were penicillin-resistant and sensible to vancomycin, quinupristin/dalfopristin and linezolid (Table 1). The prevalence of MRSA, oxacillin/methicillin resistant, was of 30% and most of the isolates were resistant to multiple antibiotics, which mean they were able to counteract different mechanisms of action (from cell wall synthesis to DNA topoisomerase inhibition). Four of them were resistant to at least seven antibiotics (oxacillin, penicillin, clindamycin, erythromycin, sulfamethoxazole/trimethoprim, rifampicin and norfloxacin). Resistance to erythromycin, sulfamethoxazole-trimethoprim and clindamycin reached 58%, 43% and 28%, respectively. Particularly among the MRSA, the prevalence of resistant to those antibiotics was very elevate (87%, 83% and 60%, respectively) in comparison to the rates reported in previous studies [20-22]. Nowadays, it is common to see such resistance profiles, especially against clindamycin, due to haphazard use of these antibiotics [22].

The prevalence of MRSA was 30%, what is alarming in a hospital environment considering that resistance can spread fast. Other studies have also reported high prevalence of MRSA in clinical samples [20-22]. Nowadays, vancomycin is used as drug of choice for treating MRSA, but the emergence of vancomycin intermediate-sensitive *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) has limited even this therapeutic route [2]. In these more severe cases, the drugs of choice are quinupristin/dalfopristin, minocycline, daptomycin and linezolid [23-28]. Taken together, these issues highlight the importance of searching new drugs to fight such pathogens.

Conclusion

In our study high rates of antibiotic resistance of clinical isolates of *S. aureus* were observed. Furthermore, our findings highlight the significant antibacterial activity of dactylomelin-P against those isolates including MRSA. Dactylomelin-P, with its alternative L-AAO-based mechanism of action, stood out with a different drug-target interaction, which is very important currently for an effective action against multiple antibiotic-resistant *S. aureus.* Further studies are necessary to assess the effectiveness of dactylomelin-P for *in vivo* applications.

References

1. Atya AKA, Belguesmia Y, Chataigne G, Ravallec R, Vacheé A, et al. (2016) Anti-MRSA Activities of Enterocins DD28 and DD93 and Evidences on Their Role in The Inhibition of Biofilm Formation. Front Microbiol 7: 1-12.
2. Kshetry AO, Pant ND, Bhandari R, Khatri S, Shrestha KL, et al. (2016) Minimum inhibitory concentration of vancomycin to methicillin resistant *Staphylococcus aureus* isolated from different clinical samples at a tertiary care hospital in Nepal. Antimicrob Resist Infect Control 5: 27.
3. Kurosu M, Siricilla S, Mitachi K (2013) Advances in MRSA drug discovery: where are we and where do we need to be? Expert Opin Drug Discov 8(9): 1095-1116.
4. Katz L, Baltz RH (2016) Natural product discovery: past, present, and future. J Ind Microbiol Biotechnol 43(2-3): 155-176.
5. Melo VMM, Duarte ABG, Carvalho AFFU, Siebra EA, Vasconcelos IM (2000) Purification of a novel antibiotic and haemagglutinating protein from purple fluid of the sea hare *Aplysia dactylomela* Rang 1828. Toxicon 38(10): 1415-1427.
6. Chapman DJ, Fox DL (1967) Bile pigment metabolism in the sea-hare *Aplysia*. Journal of Experimental Marine Biology and Ecology 4: 71-78.
7. Carew TJ, Kandel ER (1977) Inking in *Aplysia californica*. JNeurophysiol 40(3): 692-707.
8. Ambrose HW, Givens RP, Chen R, Ambrose KP (1979) Distastefulness as a defense mechanism in *Aplysia brasiliana* (Mollusca:Gastropoda). Marine Behavior & Physiology. 6(1): 57-64.
9. Gilly WF, Lucero MT (1992) Behavioral responses to chemical stimulation of the olfactory organ in the squid *Loligo opalescens*. The Journal of Experimental Biology 162: 209-229.
10. Pennings SC (1994) Interspecific variation in the chemical defenses in the sea hares (Opisthobranchia: Anaspidae). Journal of Experimental Marine Biology and Ecology 180: 203-219.
11. Nolen TG, Johnson PM, Kicklighter CE, Capo T (1995) Ink secretion by the marine snail *Aplysia californica* enhances its ability to escape from a natural predator. Journal of Comparative Physiology 176(2): 239-254.
12. Carefoot TH, Pennings SC, Danko JP (1999) A test of novel function(s) for the ink of sea hares. Journal of Experimental Marine Biology and Ecology 234(2): 185-197.
13. Kicklighter CE, Shabani S, Johnson PM, Derby CD (2005) Sea hares use novel antipredatory chemical defenses. Curr Biol 15(6): 549-554.
14. Kicklighter CE, Derby CD (2006) Multiple componentes in the ink of the sea hare *Aplysia californica* are aversive to the sea anemone *Anthopleura sola*. Journal of Experimental Marine Biology and Ecology 334(2): 256-268.
15. Love Chezem T, Aggio JF, Derby CD (2013) Defense through sensory inactivation: sea hare ink reduces sensory and motor responses of spiny lobsters to food odors. J Exp Biol 216(pt 8): 1364-1372.
16. Tavares TCL, Nogueira VLR, Vasconcelos IM, Gomes VG, da Cunha M, et al. (2011) Further characterization and mode of action of dactylomelin-P, an antibacterial protein isolated from the ink of the sea hare *Aplysia dactylomela*. J Exp Mar Biol Ecol 407 (2): 200-206.
17. Ko KC, Tai PC, Derby CD (2012) Mechanisms of Action of Escapin, a Bactericidal Agent in the Ink Secretion of the Sea Hare *Aplysia californica*: Rapid and Long-Lasting DNA Condensation and Involvement of the OxyR-Regulated Oxidative Stress Pathway. Antimicrob Agents Chemother 56(4): 1725-1734.
18. NCCLS, National Committee for Clinical Laboratory Standards (2003) Performance Standards for Antimicrobial Disk Susceptibility Tests. Approved Standard-Eighty Edition. NCCLS document M2-A8. Pennsylvania, USA.
19. Caffrey AR, Timbrook TT, Noh E, Sakoulas G, Opal SM, et al. (2017) Evidence to support continuation of statin therapy in patients with *Staphylococcus aureus* bacteremia. Antimicrob Agents Chemother 61(3): e02228-16.
20. Shrestha B, Pokhrel BM, Mohapatra TM (2009) Phenotypic characterization of nosocomial isolates of *Staphylococcus aureus* with reference to MRSA. J Infect Dev Ctries 3(7): 554-560.
21. Ansari S, Nepal HP, Gautam R, Rayamajhi N, Shrestha S, et al. (2014) Threat of drug resistant *Staphylococcus aureus* to health in Nepal. BMC Infect Dis 14: 157.
22. Belbase, A, Pant N D, Nepal K, Neupane B, Baidhya R, et al. (2017) Antibiotic resistance and biofilm production among the strains of *Staphylococcus aureus* isolated from pus/wound swab samples in a tertiary care hospital in Nepal. Ann Clin Microbial Antimicrob 16(1): 15.

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23. Rivera AM, Boucher HW (2011) Current Concepts in Antimicrobial Therapy Against Select Gram-Positive Organisms: Methicillin-Resistant S*taphylococcus aureus*, Penicillin-Resistant Pneumococci, and Vancomycin-Resistant Enterococci. Mayo Clin Proc 86(12): 1230-1243.

24. Kasai K, Ishikawa T, Nakamura T, Miura T (2015) Antibacterial properties of L-amino acid oxidase: mechanisms of action and perspectives for therapeutic applications. Appl Microbiol Biotechnol 99(19): 7847-7857.

25. Ehara T, Kitajima S, Kanzawa N, Tamiya T, Tsuchiya T (2002) Antimicrobial action of achacin is mediated by L-amino acid oxidase activity. FEBS Lett 531(3): 509-512.

26. Lee ML, Tana NH, Funga SY, Sekaran SD (2011) Antibacterial action of a heat-stable form of L-amino acid oxidase isolated from king cobra (*Ophiophagus hannah*) venom. Comp Biochem Physiol C Toxicol Pharmacol 153(2): 237-242.

27. Kitani Y, Kikuchi N, Zhang G, Ishizaki S, Shimakura K, et al. (2008) Antibacterial action of L-amino acid oxidase from the skin mucus of rockfish *Sebastes schlegelii*. Comp Biochem Physiol B: Biochem Mol Biol 149(2): 394-400.

28. Kasai K, Ishikawa T, Komata T, Fukuchi K, Chiba M, et al. (2010) Novel L-amino acid oxidase with antibacterial activity against methicillin-resistant *Staphylococcus aureus* isolated from epidermal mucus of the flounder *Platichthys stellatus*. FEBS J 277(2): 453-465.

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