Chromobacterium violaceum ATCC12472: Multi-drug and ethidium bromide resistant

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Abstract

Chromobacterium violaceum is an opportunistic human pathogen causing a range of gastric infections and occasionally septicemia. This Gram-negative bacillus is a common inhabitant of soil and water in tropical and subtropical areas of the world. Infection occurs after contamination of damaged skin exposed to soil or environmental water. Alternatively, systemic infection can follow the aspiration or ingestion of contaminated water. The major features of infections by *C. violaceum* are, in generally, rapid clinical course, multiple visceral abscesses, and high mortality. Genomic data on the type strain ATCC 12472 has provided a comprehensive basis for detailed studies of pathogenicity, virulence and drug resistance genes. In this study, the susceptibility of this organism was tested on a variety of drugs at different concentrations, in solid and liquid media. *C. violaceum* shown to be resistance to ampicilin, penicillin, rifampicin, erythromycin, vancomycin and also to ethidium bromide. The bacteria was susceptible to gentamicin, tetracycline, chloramphenicol, cotrimoxazole, kanamycin, streptomycin and nalidixic acid, at the tested concentrations.

Key words: *Chromobacterium violaceum*; multi-drug resistance; ethidium bromide.

Introduction

Chromobacterium violaceum is a common inhabitant of soil and water in tropical and sub-tropical regions. This Gram negative bacillus is considered as a saprophyte, but occasionally it can act as an opportunistic pathogen for animals and man (Crosse *et al.* 2006; Fombuena *et al.* 1998; Hassan *et al.* 1993; Ti *et al.* 1993). In immunocompromised individual the localized lesion might lead to septicaemic infections with abscesses in multiple internal organs, urinary tract infection and diarrhoea. The most common feature of this infection is sepsis, followed by cutaneous involvement and liver abscesses (Midani & Rathore, 1998). Lung abscesses have also been reported (Sirinavin *et al.* 2005; Tee *et al.* 2006; Teoh *et al.* 2006). Mortality rate is very high for patients with disseminated infections (Ang, 2004). The first case in humans was reported in Malaysia in 1927, and the medical literature contains reports of approximately 150 human cases since then (Midani & Rathore, 1998; Ray *et al.* 2004; Teoh *et al.* 2006). This bacteria is able to compete with other bacteria in the environment and possess a highly effective survival mechanism (Brito *et al.* 2004; Duran & Menck, 2001). Infection treatment is very difficult and currently there are no vaccines. A successfully treatment with ciprofloxacin and amikacin has been reported (Ray *et al.* 2004).

The resistance of prokaryotic organisms to many unrelated hydrophobic chemotherapeutic drugs is termed multi-drug resistance (MDR). A variety of MDR efflux systems are widely distributed among prokaryotic microorganisms, including pathogenic bacteria (Aeschlimann, 2003; Lewis, 1994; Lewis *et al.* 1994; Marquez, 2005). They belong to three different families of transport proteins: the major facilitator superfamily (MFS) (Saidijam *et al.* 2006; Vardy *et al.* 2004), the resistance nodulation division (RND) family (Gotoh *et al.* 1999; Piddock, 2006; Saier *et al.* 1994), and the small multidrug resistance (SMR) family of small translocases (Jack *et al.* 2000; Paulsen *et al.* 1996; Tate, 2006).

Genomic data of *C.violaceum* ATCC 12472 (http://www.brgene.lncc.br/cviolaceum/) has provided a comprehensive basis for detailed studies of pathogenicity, virulence and drug resistance genes (Brito *et al.* 2004; Fantinatti-Garboggini *et al.* 2004). The genome contains extensive but incomplete arrays of ORFs coding for proteins associated with mammalian pathogenicity, possibly involved in the occasional but often fatal cases of human *C. violaceum*

infection (Consortium, 2003). In this study, we provided experimental data of drug resistance of *C. violaceum* ATCC12472 on a variety of antibiotics, in different concentrations, in solid and liquid media. Ethidium bromide was also tested, in order to confirm the resistance to this toxic compound, as indicated by the genome data.

Material and Methods

Chromobacterium violaceum ATCC12472 was grown for 12 hours on shaking in Luria Bertani (LB) broth medium and in LB agar plates at 30^{o}C. For the susceptibility assay, fourteen different drugs, including ethidium bromide, were tested at different concentrations when *C. violaceum* were cultivated in liquid and in solid LB medium. Antimicrobial susceptibility was also tested using plate disc diffusion method. Experiments were repeated on triplicates and shown agreement.

Results

Chromobacterium vioalceum ATCC12472 strain is resistant to a variety of antibiotics and is also resistant to ethidium bromide, as shown in Tables 1-3. This organism is more sensitive to gentamycin and tetracycline at concentrations higher than 10μg/ml. It is very resistant to rifampicin, vancomicin, and ampicilin, even at concentrations higher than 130μg/ml. This strain was found sensitive to erythromycin at concentration higher than 50μg/ml when it was cultivated in LB broth. However, in LB agar plates it was resistant to erythromycin at concentrations higher than 130μg/ml.

Discussion

Chromobacterium violaceum ATCC12472, a wild type strain, showed to be resistant to ethidium bromide even at concentrations higher than 50μg/ml. It has been reported that mutantes of *Baciluus subtilis* was found to be resistant to 10 μg of ethidium bromide per ml (Bishop & Brown, 1973). *E-coli* mutants have been reported to be resistant to ethidium bromide at concentration higher than 75μg/ml, when *mdfA* was over-expressed. MdfA is a putative membrane transport protein and belongs to the MFS superfamily (Edgar & Bibi, 1997). There

are a large number of open reading frames associated with various mechanisms of drug resistance in the *C. violaceum* genome. Genes associated with bacitracin, bicyclomycin, chloramphenicol, kasugamycin, and methylenomycin were also found (Fantinatti-Garboggini *et al.* 2004), comprising a remarkable feature of this organism. Families of drug extrusion translocases have been identified in the *C. violaceum* genome, based on sequence homology. These are the MFS (major facilitator super family), SMR (small multidrug resistance), RND (resistance nodulation cell division), ABC (ATP-binding cassette), and MATE (multidrug and toxic compound extrusion) family (Fantinatti-Garboggini *et al.* 2004). EmrE, the product of the *emrE* gene homolog identified in the *C. violaceum* genome is an SMR-related protein. The most striking feature of EmrE is that it is capable of transporting substrates of widely varying structure, provided that overall the compound is highly hydrophobic and carries a positive charge. EmrE couples the extrusion of toxins to the influx of protons down their electrochemical gradient, with probably two or more protons transported per toxin molecule (Arkin *et al.* 1996; Lebendiker & Schuldiner, 1996; Schuldiner *et al.* 1996; Venkatraman *et al.* 2002; Yerushalmi *et al.* 1996). Presence of a large number of open reading frames associated with various mechanisms in *C. vioalceum* genome may help to explain the resistance of this organism to ethidium bromide and to a variety of antibiotics tested.

Conclusion

In this study, we have tested drug resistance of *C. violaceum* ATCC12472 strain at different concentrations of different antibiotics. *C. violaceum* is resistance to a wide broad range of antibiotics, and was found to be resistant to ethidium bromide at concentrations higher than 50μg/ml. The presence of multi-drug resistance genes found in *C. violaceum* genome may explain such ability to survive, and may represents a challenging for infection treatment.

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Table 1. Test of Antibiotics resistance to *Chromobacterium violaceum* (ATCC12472) in disc plate.

Antibiotic	**Concentration (μg/ml)**	***Susceptibility of C. violaceum ATCC12472C.***
Ampicilin	10	+
Penicilin	10	+
Gentamicin	10	-
Tetracycline	30	-
Chloramphenicol	30	-
Cotrimoxazole	25	-

* (+) Resistant; Sensitive(-).

233 Table 2. Test of antibiotics resistance to *Chromobacterium violaceum* (ATCC12472) in solid

234 media (LB agar)

Antibiotic	**Concentration (μg/ml)**	***Susceptibility of C. violaceum* ATCC12472**
Rifampicin	10	+
	20	+
	30	+
	50	+
	100	+
	150	+
	200	+
Erythromycin	50	+
	100	+
	150	+
Ethidium bromide	10	+
	15	+
	20	+
	25	+
	40	+

235 * (+) Resistant; Sensitive(-).

236 Table 3. Test of antibiotics resistance to *Chromobacterium*

237 *violaceum* (ATCC12472) in liquid media (LB broth).

Antibiotic	**Concentration (μg/ml)**	***Susceptibility of *C. violaceum* ATCC12472**
Ampicilin	50	+
	100	+
	150	+
Tetracycline	10	-
Kanamycin	50	-
Chloramphenicol	50	-
Streptomycin	50	-
Nalidixic Acid	15	-
Vancomycin	50	+
	75	+
	100	+
	150	+
Erythromycin	50	+
	75	-
	100	-
	150	-
Rifampicin	50	+
	100	-
	150	-
	200	-
Carbamycin	20	+
	50	+
	100	-
	150	-
Ethidium bromide	1	+
	10	+
	30	+
	60	+

238 * (+) Resistant; Sensitive(-).