INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

COMPARATIVE STUDY ON THE EFFECT OF CHLOROXYLENOL AND SODIUM CHLORIDE ON *BRUCELLA* AND *ACINETOBACTER* GROWTH

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ABSTRACT
A study was carried out to investigate the effect of chloroxylenol and sodium chloride on the growth of Brucella *spp.* and *Acinetobacter baumannii* isolates. Results revealed that chloroxylenol was more effect on growth of Acinetobacter *sp.* As compared with Brucella *spp.* The inhibition of the *Acinetobacter* growth was detected at all concentrations (3, 30, 300, 3000, 30000, and 40000) Mg/ ml. Although, *Brucella* isolates resist at 3 and 30 Mg/ ml of chloroxylenol, high growth inhibition was seen at 300 Mg/ ml of chloroxylenol. On the other hand, no difference was found in the effect of chloroxylenol on *Brucella abortus* and *Brucella melitensis*. The results confirmed that the *Brucella spp.* seems to be more susceptible to negative effect of high concentration of NaCl than *A.baumannii* .

KEYWORDS: *Acinetobacter* , *Brucella* ,Sodium chloride , Chloroxelenol.

INTRODUCTION

Infections disease account for about half of the death in tropical countries (Khosravi and Behzadi, 2006). Besides , incidents of epidemic due to drug resistant microorganisms pose enormous public health concerns (Burt and Reinders, 2003; Meraj *et al*., 2014) . Within the genus *Brucella* there are closely related species that have recognized from many years, this important group causes brucellosis in domestic animals and humans, infection by localized in reticuloendothelial tissues , reproductive organs and less frequently bone and joints (AL-Rodhan, 2005). Microbial resistance to antibiotics especially among *Brucella* species is a major threat to public health (Lang *et al*., 1995) , mainly in some developing countries like Iraq (Rhaymah *et al*., 2010). On the other hand *Acinetobacter spp.* is apleomorphic aerobic gram negative bacillus that is widely distributed in nature and causes a wide spectrum of nosocomial infections (Hrenovic and Ivankovic, 2009). It has a capacity for long term survival (up to several months) on most environmental dry surfaces and it has been identified in environmental sampling at military treatment facilities along the Tigris River in Iraq and Kuwait city. Infections in soldiers, shows an increased prevalence of multidrug resistant of *Acinetobacter spp.* in Iraq (Davis *et al*., 2005) . Antiseptics and disinfectants are used extensively in hospitals and other health care setting for a variety of hard surface applications, they are on essential part of infection control practices and aid in the prevention of nosocomial infections (Hendry *et al*., 2009). Most important factors affecting the survival of enteric bacteria are salinity, nutrient availability, microbial antagonism and antibiotic substances, and bacteria which can tolerate the high salt concentrations up to approximately 10% of sodium chloride are called osmototerat (Nester *et al*., 2004). This study aimed to investigate the antimicrobial effect of chloroxylenol solution (different concentrations) and compares its effects on *Brucella spp*. and *Acinetobacter spp*. isolates and the survival of two heterotrophic bacteria in conditions of high salt concentration .

MATERIALS & METHODS

Bacterial strains and cultuer media

Brucella abortus, Brucella melitensis and *Acinetobacter baumannii* isolates were obtained from clinical specimens with brucellosis infections and urinary tract , surgical wound infections or non surgical wound infections in Baghdad hospitals (table-1). The isolates were identified biochemically (biotype analysis) in addition to API-20E analysis (Atlas *et al*., 1995). The isolates were streaked on trypticase soy agar from Himedia (India). For short time preservative at 4 C^o and store in 20% glycerol in Brain heart infusion broth (Himedia – India), - 20C^o for long time preservative.

STUDY THE INHIBITION OF BACTERIAL GROWTH BY CHLOROXYLENOL COMPOUND.

Preparation of inoculums

The isolates subcultured on Brain heart infusion agar (Himedia – India), for 18- 24 hrs at 37C^o, culture purity was checked according to the procedure mentioned by (Forbes *et al*., 2002). Ahomogenous bacterial cell suspension (2 x 10^2) CFU \ml was prepared with turbidity equal to 0.5 Mcfarland (Baron *et al*., 1994).

TABLE 1: The prevalence of Acinetobacter baumannii and Brucella spp. isolates identified in different clinical cases

Type of isolated bacteria	No. of isolates	Type of sample
Brucella melitensis	2	Blood from brucellosis patients
Brucella abortus	3	Blood from brucellosis patients
Acinetobacter baumannii	5	Wounds infection
Acinetobacter baumannii	5	Urine from urinary tract infection patients
Total No. of isolates	15	

Chloroxylenol solution

Chloroxylenol is the principal ingredient in Dettol. It prepared by diluting with distilled water, solution of (0.2) % Chloroxylenol of commercial grade , adding to distilled water and adjusting to the final strength of Dettol to (3, 30, 300, 3000, 30.000 and 40.000)Mg\ml.

Minimal inhibitory concentration

The effect of Chloroxylenol dilutions on the growth of *Brucella* and *Acinetobacter* isolates was determined by a macrobroth dilution method (Messager *et al*., 2001).Each tube contained 1ml of 6 concentretion of Chloroxylenol (5:1) as doubling dilution in the range (3 – 40.000) Mg \ml .

The tubes were inoculated with 100 *Ml* of an 18- 24 hrs culture diluted to yield afinal inoculum of 10^8 CFU \ml. Brain heart infusion broth was used both as diluent and as culture medium. The MICs value Mg \ml was recorded as the lowest concentration in which no visible growth was apparent after incubation for 24 hrs at $37C^o$.

INHIBITION OF BACTERIAL GROWTH BY SODIUM CHLORIDE

Preparation of inoculum was done as in (Burt and Reinders, 2003; Khosravi and Behzadi, 2006). Experimental procedures as in (Hrenovic and Ivankovic, 2009).

Nutrient broth obtained from himedia – lndin was used and analytical sodium chloride (BDH – England) were added in the flasks to obtain the concentration of (1, 2 , 3 , 4,5, 6, 7, 8, 9, 10, 11) % of NaCl in nutrient broth . The pH value of broth media was adjusted to 7.0 $-^{+}$ 0.1 using pH – meter.

One ml of rhesus pended biomass was inoculated into Erlenmeyer flasks with ranging concentrations of NaCl in 100 ml of nutrient broth. The flasks were sealed and in cubated for 24 hrs in $37C^o$. starting number of viable count were determined before incubation. And the number of viable cells in each bottle was determined after 24 hrs. of incubation as colony forming units. A 1 ml of bacterial suspension was aseptically taken from each bottle and serially diluted (10^{-1},10^{-2} ,10^{-3}). Volumes of 0.1 ml were then aseptically inoculated on to nutrient agar plates (spreed –plate method). After the incubation 24 4hrs. The bacterial colonies were counted and the number of cells was reported as CFU/ml . All measurements were done in triplicate and mean values are presented.

RESULTS & DISCUSSION

The results shows that the antibacterial activity of the chloroxylenol product varies with both organisms (*Brucella spp.* and *Acinetobacter sp*.) . This variation is particularly observable against *Acinetobacter baumannii* and to a lower extent against *Brucella spp*. while virtually no variation is appear with *B. melitensis* and *B.abortus*. The MIC of the chloroxylenol against these organisms are listed in Table (2). Chloroxylenol range between (3-40000) Mg/ml for *A. baumannii* , while it range between 30 to 300 Mg/ml for *B. abortus* and *B. melitensis* , since preliminary studies usually show MIC of $\leqq$ 1 Mg/ml (Hassain, 2011), but against *streptococcus mutant* but not against this study organisms. Chloroxylenol as phenolic compound can inhibit the Adenosine triphosphatase (ATPase) which is an important enzyme that is linked to cytoplasmic membrane and thus can inhibit the process of returning potassium ions into cells in exchange for sodium and hydrogen ions, (Autio, 2008) also inhibits metabolic enzymes such as phosphoenol pyruvate phosphotransferase, which lead to inhibit and kill the organisms (Axmann and Brecx, 2008).

TABLE 2 : In vitro activity of choloroxylenol as different concentration against *Brucella spp*. and *Acinetobacter baumannii* mention as MIC.

Type of isolates	Conc. of Chloro. (mg/ml)					
	3	30	300	3000	30000	40000
B. melitensis	+	-	-	-	-	-
B. Abortus	+	-	-	-	-	-
A. baumannii	-	-	-	-	-	-

- : No growth, + : growth

In the second set of experiments nutrient rich medium was used for bacterial growth. In the control reactors without addition of Nacl , both bacteria (*Brucella* and *Acinerobacter*) were intensively multiplying and the number of both bacteria after 24 hrs increased for one order of magnitude when compared to starting number (3.75×10^2 CFU/ml) table – 3 . The bacteria most likely similar growth trend was obtained in reactors containing (1,2,3,4,5) % of NaCl . While at (6, 7, 8 , 9 and 10) % of Nacl the number of *A. baumannii* was lowed when compared to starting number and decreased further in 10 % with no growth at 11% of Nacl. Indicating decay of cells for *Brucella spp.* the growth at different concentration of NaCl was similar to that obtained for

A. baumannii. Concerning, *Brucella spp*. there was no growth at (8, 9, 10) % of Nacl. Based on the results, it seems that *Brucella spp*.was more susceptible to negative effect of high concentration of Nacl than *A. baumannii*. Increased concentration of NaCl caused a failure in bacterial multiplication and decay of bacterial cell and death of all cells in the growth media . These finding agree with Stein(2000).

TABLE 3: The effect of sodium chloride on the growth of *Brucella spp.* and Acinetobacter *baumannii*

Type of isolates	No. isolates	Concentration of Nacl % CFU/ ml × 10^2											
		1	2	3	4	5	6	7	8	9	10	11	Start No.
A.baumannii	10	3.60	3.59	3.50	3.35	3.35	3.00	2.30	1.60	1.10	0.8	0	3.75
B.abortus	3	3.21	2.48	1.77	1.06	0.35	0.7	0	0	0	0	0	3.26
B. melitensis	2	3.50	3.28	3.15	2.13	0.71	0.15	0.2	0	0	0	0	3.58

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