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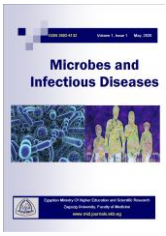

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Original article

Insights into the prevalence and the antimicrobial activity of natural product against *Pantoea* spp in neonatal intensive care unit

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Background: Neonatal sepsis is one of the important causes of morbidity and mortality in a neonate. *Pantoea species (Pantoea spp*) has attracted considerable attention as an emerging neonatal pathogen and has been associated with life-threatening septic arthritis, occupational respiratory infections, and bloodstream infections in neonates and infants globally. **Methods:** We aimed to determine the prevalence of Pantoea spp from neonatal sepsis cases, and the sources of its infection. Pantoea spp isolates were isolated and identificatied by conventional methods then, they were confirmed by VITEK 2 and conventional PCR. Furthermore, we evaluated the antimicrobial profile of *Pantoea spp*. Also we studied the antimicrobial effect of anise oil and peppermint oil by using the MIC method, and hibiscus and lactoferrin by using the cup diffusion method. **Results:** we reported three cases (6%) of neonatal sepsis caused by the emerging life-threatening pathogen *Pantoea spp* in the NICU of Assuit University. The organism was also detected in contaminated powder infant formula (PIF) and parentral nutrition (PN) in 16% and 2%, respectively which may be sources of infection for neonates. **Conclusion:** PIF and PN may by source of *Pantoea spp* infection. Anise oil , peppermint oil , hibiscus and lactoferrin have antimicrobial effect against *Pantoea* spp.

Introduction

The genus *Pantoea* was derived from the Greek term "Pantoios," which means "of many sorts or sources," This demonstrates the variety of bacteria that can be extracted from different ecological and geographic contexts [1].

Recently, the *Pantoea* genus has 20 identified species, all of which are phenotypically similar and can only be distinguished from one another using multilocus sequence analysis (MLSA) of housekeeping genes [1].

Pantoea species*(Pantoea* spp.*)* is one of the pathogens responsible for newborn sepsis due to their immunodeficient state and prematurity, they are at a higher risk of developing hospital-acquired infections [2]. Since 1970, *Pantoea* infections have been linked to epidemics in healthcare facilities in the USA [3]. *Pantoea* infection was identified in

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125 patients (2%) without clustering in a survey of 6,383 patients performed in the Netherlands between January 1994 and June 2005 [4]. Another case series reported that five out of 1,665 newborn children admitted to two different NICUs acquired nosocomial bloodstream infections caused by *Pantoea* in Kuwait from January 2005 to December 2006 [5]. Likewise, *Pantoea* was isolated in 34.7% (8/23) in a pediatric unit from a single-center study in Turkey (2000-2015) [6].

Since it has been isolated from a variety of ecological niches and hosts, including plants, animals, insects, and humans, the *Pantoea* genus is a diverse group in the *Enterobacteriaceae* family [7]. *Pantoea* spp. were frequently found attached to plants as diseases or epiphytes. This organism is a newly emerging opportunistic pathogen. This bacteria typically infect immunodeficient hosts or newborns [8]. They may also be linked to secondary bacteremia or nosocomial infections that are connected to medical equipment like intravenous catheters or tainted intravenous fluids, as well as trauma brought on by plant material penetration when conducting agricultural tasks, gardening, or children's play [9].

Only newborns and young children are susceptible to diseases brought on by foodborne microorganisms. Neonatal illness development will be linked to *Pantoea agglomerans* contamination of powdered infant formula. Therefore, the microbiological safety of powdered infant formula milk (PIF) is of the most importance. PIF is a fantastic medium for bacterial growth because it is not a sterile substance. The primary components of PIF are bovine milk and plant matter, which are also potential sources of several microorganisms that are harmful to both children and adults [10].

Materials and methods

Study design

A cross-sectional, descriptive study from June 2019 to June 2020 was done in the neonatal intensive care unit (NICU) of Assiut University Children's Hospital in Egypt after being approved by the Medical Ethics Committee, Faculty of Medicine, Assiut University (IRB no. 17300296).

Patients

Neonates with suspected neonatal sepsis complaining of lethargy, seizures, fever or hypothermia, high C-reactive protein (CRP), changes in feeding patterns, and symptoms of respiratory distress such as tachypnea (respiratory rate, >60/min), significant chest indrawing, grunting, and cyanosis were included in this study [11]. Blood cultures were taken and examined. Patients with negative blood cultures were excluded.

A thorough clinical examination including measurements of their temperature, breathing, color, presence of lethargy, neurological problems, and feeding patterns, a complete blood count, and C-reactive protein assessment was performed.

Sampling

This study included 50 venous blood samples taken from neonates with sepsis for blood culture, 50 powdered infant formula milk (PIF), and 50 parenteral nutrition (PN) samples which previously opened to be consumed in NICU for isolation and identification of *Pantoea* spp.

Blood culture

Blood was aseptically collected from a peripheral vein and directly inoculated into BacT/ALERT blood culture bottles (bioMérieux, Marcy L'Etoile, France). The BacT/ALERT 3D equipment (bioMérieux, Marcy l'Etoile, France) was used to monitor the results. The bottles were incubated for seven days at 30 C°, after which positive specimens were inoculated onto the blood, MacConkey, and violet red bile glucose (VRBG) agars and incubated for 24 hours at 30 C° [12].

Isolation and identification of *Pantoea* spp. from powdered infant formula milk (PIF)

Isolation was performed basically according to the U.S. FDA isolation and enumeration method, 900 ml of distilled water was combined with 100 g of PIF before being gently mixed and incubated for 24 hours at 30 C°. 90 ml of *Enterobacteriaceae* Enrichment (EE) broth was mixed with 10 ml of the pre-enrichment mixture, and the mixture was then incubated at 30 C° for 24 hours. A loopful of the enrichment culture was streaked onto duplicate (VRBGA) plates after incubation, and the plates were then cultivated at 30 C ° for 18–24 hours [10].

Isolation and identification of *Pantoea* spp. from parenteral nutrition (PN)

Regarding PN, trypticase soy broth (TSB) and brain heart infusion (BHI), respectively, were inoculated with one milliliter of PN solution. After 24 hours of incubation at 30 C, they underwent another 24 hours of incubation at 30 C with the addition of 10 ml of the enrichment mixture to 90 ml of *Enterobacteriaceae* Enrichment (EE) broth. A

loopful of the enrichment broth was smeared onto duplicate (VRBGA) plates and cultured at 30 C° for 18-24h [13].

Confirmation of *Pantoea* spp. isolates by VITEK 2 and conventional PCR

Pantoea creates purple/pink colored colonies on VRBGA agar which was reported as a selective media for *Pantoea* spp. Also, it gives convex, smooth, punctuate, and umbilicated lactose fermenting glistening colonies on MacConkey agar as described by **Mardanh et al.** [10]. The colonies suspected to be of Pantoes were further confirmed using VITEK 2 automated microbiology system (VITEK 2 GN ID card, bioMeriéux's) at the department of Clinical Pathology, Faculty of Medicine, Assiut University. Also, the conventional PCR was used to confirm *Pantoea* spp. using the primers:
PANsp_atpD_fwd:5'GAGGGTAACGACTTCTA CCAC3' and PANsp_atpD_rev: 5'CTGTACGGAGGTGATTGAAC 3' (Invitrogen, United States) [1].

PCRs were performed using the thermocycler model (simpliAmp, Applied Biosystems, USA). The amplification reactions were carried out at a defined total volume of 50 µL. The amplification procedure included initial denaturation for 3 min at 94 °C, then 30 cycles were achieved, each consisting of denaturation for 30 s at 94 °C, annealing for 30 s at 58 °C, and extension for 2 min at 72 °C and a final extension of 10 min at 72°C. PCR product was examined by electrophoresis in 2% (w/v) agarose gel as described by **Kini et al.** [1]. The gel was then examined under ultraviolet light, and the result in the PCR product was measured against a standard DNA ladder (50 base pair ladder) when appearing band 330 bp that indicated *Pantoea* spp [1].

Antibiotic sensitivity test

Isolated colonies of *Pantoea* spp. were examined for their sensitivity against 16 antibiotics using the modified Kirby–Bauer disc diffusion method. comparing results with CLSI 2021 guidelines [14].

Studying the antimicrobial effect of anise oil, peppermint oil, hibiscus, and lactoferrin against isolated *Pantoea* spp.:

Determination of minimum inhibitory concentrations (MIC) values of peppermint oil and anise oil

The MIC of anise oil and peppermint oil was determined by microdilution according to CLSI 2021[14,15]. Serial twofold dilutions were performed ranging from 4 to 0.0125% (v/v) from essential oil (EO) prepared in trypticase agar with 0.6 % yeast extract supplement (TSAYE). The greatest dilution of samples (anise oil and peppermint oil) without detectable growth after 24 hours of incubation at 30 °C was regarded as the MIC. For this study the positive control agent was gentamicin 10 µg/mL. Resazurin dye was utilized for accurate MIC determination. MIC was defined as the lowest amount of EO that prevented the color change from blue to pink [16].

Agar well diffusion assay for evaluating the antimicrobial activity of hibiscus and lactoferrin against *Pantoea* spp.:

Pantoea spp. colonies were directly suspended in 0.85% sterile saline from (VRBGA) plates to achieve turbidity similar to that of the 0.5 McFarland standards (about 10^8 CFU/ml). Aliquots (100µl) of the solution were applied to the surface of sterile (VRBGA) plates and left there for 24 hours at 30 C. It employed the agar well diffusion technique bioassay [17].

Each (VRBGA) plate was previously swabbed with *Pantoea* spp. culture, and 0.5 cm wells with a diameter of that size were created using a sterile cork borer. The prepared solutions (hibiscus aqueous extract, lactoferrin, and positive controls gentamicin 10 µg/mL) were then added to the cut wells in 100 µl. Plates were kept for 30 minutes at room temperature to allow diffusion of substances and then were incubated at 30C° for 24 hours. antimicrobial activity was determined by measuring the diameter of the inhibition zone in mm and comparing results with CLSI 2021guidelines [14].

Statistical analysis

Statistical analyses were performed using the statistical package for social sciences, version 16.0 (SPSS Inc., Chicago, IL, United States). Data are represented as the mean ± SD for continuous variables and as percentages for categorical variables, Also the results of the conventional culture for *Pantoea* spp. identification were compared to the results from PCR of 16s rRNA gene.

Results

The clinical and demographic characteristics of neonates included in the study are summarized in **table (1)**. *Pantoea* spp. was isolated from 3 out of 50 culture-proven sepsis, giving a prevalence rate of 6%.*Pantoea* spp. was detected in 8/50 PIF samples and 1/50 of PN samples.

The results of the 16 antibiotics used in the sensitivity testing performed for the positive cases derived from PIF, intravenous solutions, and blood are shown in **table (2**). *Pantoea* spp. was highly resistant to imipenem, nalidixic acid, and ampicillin. On the contrary, *Pantoea* spp. was sensitive to tetracycline, gentamycin, and chloramphenicol.

The results of the antimicrobial effect of anise oil and peppermint oil on *Pantoea* spp. by determination of minimum inhibitory concentrations (MIC) values were summarized in **table (3),** which showed that anise oil and peppermint oil have an antimicrobial effect against *Pantoea* spp.

Also, we studied the antimicrobial effect of lactoferrin and hibiscus on *Pantoea* spp. isolates by cup diffusion agar method. Results are shown in **table (4)**

Table 1. Demographic and clinical data of the study population.

Variable	Number of cases (%)
Male gender	30(60%)
Female gender	20(40%)
Birth weight (g)	2,000 (1,000–3,000)
Cesarean section	42 (84%)
Low Apgar score at 1 min	33 (66%)
Low Apgar score at 5 min	12 (24%)
Central line insertion	30 (60%)
Maternal risk factors	
Preeclampsia	4 (8%)
Premature rupture of membranes	3 (6%)
Fever	3 (6%)
Antepartum hemorrhage	1 (2%)
Clinical manifestations	
Enteral feeding	
By oral	
By Ryle	7(14%)
Fever >37.8_C	36 (72%)
Hypothermia <36_C	20 (40%)
Jaundice	7 (14%)
Respiratory distress	16 (32%)
Apnea	44 (88%)
Pneumonia	10 (20%)
Diarrhea	17 (34%)
Elevated CRP	14 (28%)
Total leukocytic count (cells/ml)	50 (100%)
Mean _ SD	11.38_22.97
Median (range)	23.85 (2.9–49.0)
Absolute neutrophilic count (<1,000 cells/ml)	4 (8%)
Platelet count (<100 cells/ml)	12 (24%)

The Apgar score comprises five components: heart rate, respiratory effort, muscle tone, reflex irritability, and color, each of which is given a score of 0, 1, or 2. A low Apgar score means a score less than 7 at 1 and 5 min (American Academy of Pediatrics, 2006). Jaundice is a yellow discoloration of the sclera. Respiratory distress symptoms include tachypnea, grunting, nasal flaring, and intercostal retractions. Pneumonia is evident on a chest X-ray.

Table 2. Antimicrobial susceptibility pattern of *Pantoea* spp. isolates.

Antimicrobial drug	No. of resistant isolates%	No.of intermediate isolates%	No.of sensitive isolates%
Imipenem	12 (100%)	0	0
Nalidixic acid	12 (100%)	0	0
Ampicillin	12 (100%)	0	0
Meropenem	0	2 (16.66%)	10(83.33%)
Kanamycin	0	2 (16.66%)	10 (83.33%)
Tetracycline	0	0	12 (100%)
Gentamycin	0	0	12 (100%)
Tazobactam/piperacillin	10 (83.33%)	2 (16.66%)	0
Ceftriaxone	9 (75%)	3(25%)	0
Cefixime	10 (83.33%)	2 (16.66%)	0
Amikacin	0	2 (16.66%)	10 (83.33%)
Trimethoprim/ sulfamethoxazole	0	1 (8.33%)	11 (91.66%)
Chloramphenicol	0	0	12 (100%)
Streptomycin	0	1 (8.33%)	11 (91.66%)
Aztreonam	0	0	12 (100%)
Ciprofloxacin	10 (83.33%)	2 (16.66%)	0

Table 3. Determination of minimum inhibitory concentration (MIC) of anise oil and peppermint oil against *Pantoea* spp. isolates.

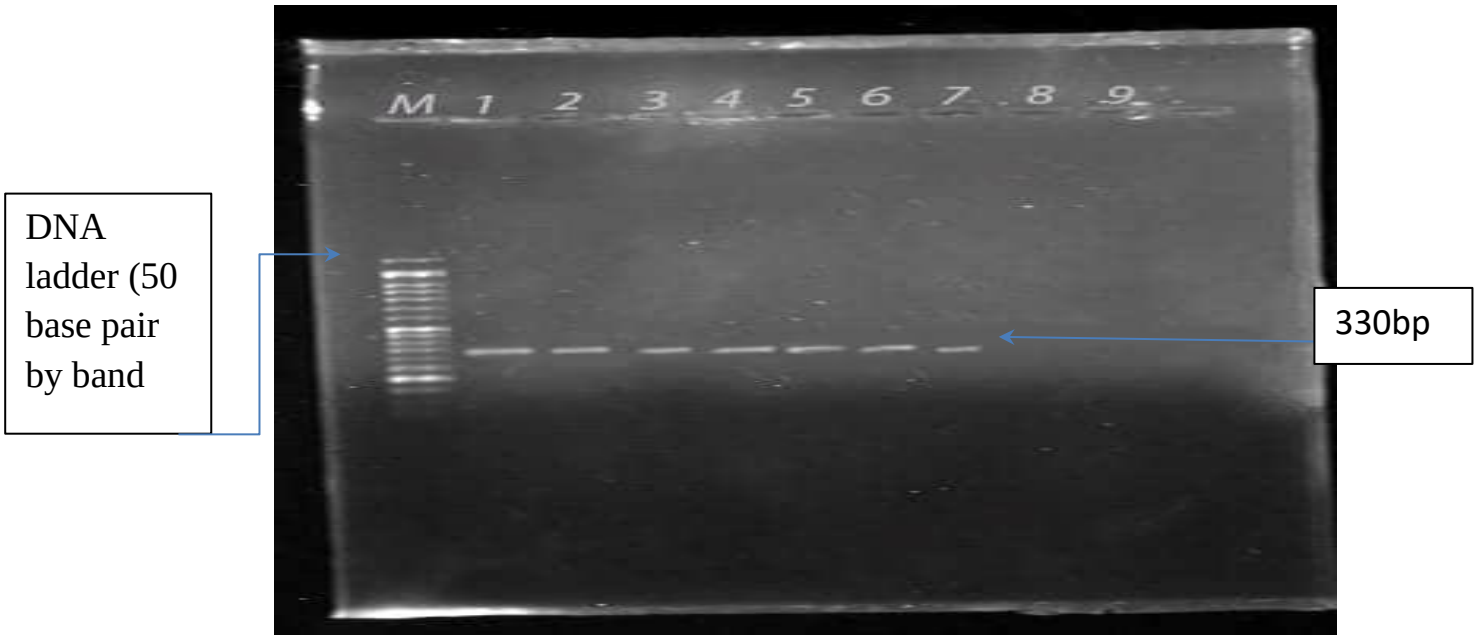
Strains	Antimicrobial agents	Final EO addition to TSAYE % (V/V)				
		4	2	1	0.5	0.25
Pantoea spp.	Anise oil	-	-	-	+	+
Pantoea spp.	Peppermint oil	-	-	-	-	+

-ve: No bacterial growth.
+ve: Bacterial growth.
EO: Essential oil.
TSAYE: Trypticase soya agar with 0.6 %yeast extract supplement.

Table 4. Inhibition zone (mm) of lactoferrin and hibiscus against *Pantoea* spp. isolates.

Samples	Inhibition zone in (mm) (Mean ± SD)
Water extract of hibiscus	(29±2)
Lactoferrin (20mg/ml)	(19± 2)
Gentamycin (10µg/ml)	(29±2)

Figure 1. Gel electrophoresis of the PCR amplified products for the detection of 16s rRNA of Pantoea spp. lane M is a DNA marker 50 base pair by a band, lanes 2 to7 are represented to positive isolates of 16s rRNA gene (330bp), lane 1 is positive control obtained from the Department of Clinical Pathology Assiut university and lane 9 is negative control.



Discussion

Pantoea spp. is one of the bacteria responsible for the largest nosocomial outbreaks in the United States in 1970–1971, with 378 cases of septicemia. Noteworthy to mention that *Pantoea agglomerans* was the only organism identified in 152 out of 378 of these cases and complicated by about a 13.4% fatality rate. The contamination of threaded screwcaps on bottles of parenteral solutions for intravenous injections caused the *Pantoea* epidemic [3]. Additionally, a septicemic outbreak caused by *Pantoea* spp. infection of parenteral feeding resulted in Malaysia and involved neonates in intensive care with a high fatality rate (87.5%) [18].

In our investigation, *Pantoea* spp. was isolated from 3 out of 50 culture-proven sepsis (6%). After receiving multiple treatments, the clinical signs improved in two neonates out of the three *Pantoea* spp. cases. One case, though, had a markedly compromised mental and physical state and passed away two weeks after admission.

On the other hand, Six cases of *Pantoea* spp. sepsis were reported by **Focà et al.** [19] in a Calabrian University Polyclinic in Italy in 2006, and six pediatric patients in Brazil were the victims of a nosocomial epidemic of the disease in 2007 that was brought on by contaminated intravenous hydration tubing, according to **Bicudo et al.** [20].

Blood is the most typical sample from which *Pantoea* is isolated in newborns. The respiratory tract and central venous catheters are the most common sources of *Pantoea* bacteremia. Skin infections, peritonitis, and urinary tract infections are additional causes of *Pantoea* bacteremia [21].

Another case study reported that between January 2005 and December 2006 in Kuwait, five out of 1,665 newborn children admitted to two distinct NICUs developed nosocomial bloodstream infections caused by *Pantoea* [5]. Additionally, in Brazil, *Pantoea* spp bacteremia was reported in seven patients receiving hemodialysis or plasmapheresis due to contamination of an anticoagulant solution made by the hospital pharmacy, according to **Boszczowski et al.** [22], **Izzo et al.** [23] reported seven cases of septicemia *Pantoea* spp. in oncologic patients, caused by infected catheters at Iseo Hospital, Brescia (Italy).

Neonatal infections made up 34.7% (8/23) of the *Pantoea* isolates in a single-center study from Turkey that examined the clinical and microbiological aspects of Pantoea infections in pediatric patients from 2000 to 2015 [6]. CDC reported that contaminated PN and intravenous fluid have been reported to cause sepsis outbreaks. The contamination may have been caused by mistakes

made when the pharmacy compounded the PN or the solutions [24].

In this study, *Pantoea* spp. *were* isolated in one sample of 50 previously opened and consumed PN samples at a percentage of 2%, While **Van Rostenberghe et al.** [18] and **Habshah et al.** [13] reported the same epidemic of newborn sepsis among eight neonates caused by *Pantoea* spp. that was spread by tainted parenteral nutrition (PN) solutions from a tertiary care hospital in Malaysia.

Infant formula made from powdered milk is not a sterile product and could be intrinsically or externally contaminated with different germs that can lead to serious illnesses in young children [10]. In this study, 16% (8/50) of the previously opened and consumed PIF in the NICU included *Pantoea* spp. This result was greater than the research conducted in Iran in 2013 by **Mardaneh et al. [10]**, who isolated *Pantoea* spp. from 125 (PIF) samples fed in NICU at a rate of (6.4%).

Numerous studies have shown that antibiotics can successfully eradicate *Pantoea* spp, although extended antibiotic use is not recommended as it may lead to the emergence of *Pantoea* antibiotic resistance [25].

Early-generation penicillin, early-generation cephalosporins, broad-spectrum cephalosporins, antipseudomonal penicillins, fluoroquinolones, aminoglycosides, trimethoprim-sulfamethoxazole (TMP-SMX), and tetracyclines are only a few of the drugs that *Pantoea* spp. has demonstrated resistance [2]. It is well recognized that using broad-spectrum antibiotics increases the danger of colonization in healthcare facilities and the evolution of antibiotic resistance. The colonization of the gastrointestinal tract may serve as an organism reservoir [26]. In our study, *Pantoea* spp. isolates showed high resistance (100%) to imipenem, nalidixic acid, and ampicillin. Variable resistance was shown to piperacillin with tazobactam, ceftriaxion, and ciprofloxacin at a percentage of (83.33%) and to cefixime at a percentage of (75%).

According to **Mardaneh et al.** [10] 62.5% of the isolates were resistant to carbenicillin, 87.5% to ampicillin, piperacillin, and mezlocillin, while 50% of the isolates were resistant to cefotaxime, moxifloxacin, co-trimoxazole, and ticarcillin. Although *Pantoea* spp. was discovered to be resistant to ampicillin and cefazoline in a 2009 study by **Liberto et al.** [27]. Over 15 years in pediatric patients, the antimicrobial susceptibility of *Pantoea* spp. revealed that 21.4% of isolates were resistant to carbapenem [6].

Adam and Darweesh [25] reported that *Pantoea* spp isolates were highly resistant to amoxicillin(90%), and gentamicin (87.5%). While the resistance to cephalothin (57.5%), cefotaxime (57.5%), and ciprofloxacin (52.5%) was different. In contrast, we observed in our study that *Pantoea* spp was highly sensitive (100%) to gentamycin, tetracycline, chloramphenicol, and aztreonam while it was sensitive to TMP-SMX and streptomycin at a percentage of (91.66%) and sensitive to amikacin, meropenem, and kanamycin at a percentage (83.33%)**. Liberto et al.** [27] had similar observations for susceptibility to meropenem recorded by **Tiwari et al**. [28] who treated successfully neonatal sepsis with intravenous Meropenem.

Strong antifungal and antibacterial properties can be found in essential oils (EO), which are extracted from a variety of plants. Additionally, several studies noted that EOs have potent antibacterial effects on microorganisms [29]. In this study, we studied the antibacterial effect of anise oil and peppermint oil against *Pantoea spp* by using minimal inhibition concentration (MIC) methods. The results showed that the MIC of Anise oil was 1% **(**v/v) while the results obtained by Topuz et al. [30] were 0.75 % (v/v). On the other hand, the MIC of Peppermint oil was 0.5 (v/v)% This result is correlated well with those obtained by **Liang et al. [31].**

The peppermint oil, which contains pulegone, menthone, menthol, carvone, 1, 8-cineole, limonene, and b-caryophyllene as well as phenolic chemicals like -pinene, citronellol, and methyl eugenol, is thought to be responsible for its antibacterial properties [32]. The lipophilic components of peppermint oil may easily flow through cell membranes, acting as an antibacterial agent by rupturing the membrane and killing germs [32].

The main compound of anise is anethole, which is characterized by amphiphilic properties, which permit the interaction with the cytoplasmic membrane, membrane fluids, proteins, lipids, and other molecules vital to microbes cells [33]. While **Dorman and Deans** [34] showed in their study that β-linalool and eugenol were responsible for the antibacterial activity which find in anise oil.

Medicinal plants continue to play a central role in the healthcare systems, especially in

underdeveloped nations where the use of herbal medicine has a long and continuous tradition**.** The discovery of multidrug resistance in several reports on dangerous bacteria in humans and animals, together with an unfavorable antibiotic side effect, has sparked intense interest in the quest for new antimicrobial medications with plant origins [35].

Hibiscus surattensis L.calyces essential oil was found to have a stronger antibacterial impact against a variety of foodborne pathogens in the **Akarca** [36] investigation. It is estimated that the antibacterial effect is due to the composition of the calyces essential oil such as β-caryophyllene, menthol, methyl salicylate, camphor, and germacrene. In this study, we estimated the antibacterial effect of watery extract hibiscus against *Pantoea* spp. and recorded inhibition zones ranging from (27mm to 31mm). These results are consistent with that of **Al -Alak et al.** [37] who found that watery extracts of hibiscus recorded inhibition zones ranging from 10mm to 32 mm in five bacterial species, including *Enterobacter* spp.

The mucosal immune system and body have a key place for lactoferrin. The mucosal tissue is the first system to adhere to and respond to microbial pathogens due to lactoferrin production. It prevents the growth of fungi, viruses, and both Gram-positive and Gram-negative bacteria**.** It has the ability to bind to free iron, which is necessary for bacterial development and causes lactoferrin to have a bacteriostatic effect. Lactoferrin is an essential protein that can prevent the growth of harmful bacteria in the stomach and limit tissue or cell damage [38]. In this study, we studied the antibacterial effect of lactoferrin (20mg/ml) on *Pantoea* spp. in vitro, which gave an inhibition zone (19±2) mm. **Hameed** [39] indicated that lactoferrin at a concentration of 20mg/ml had a maximum inhibitory effect against *Cronobactar sakazakii*. and concluded that bovine lactoferrin may have potential usefulness for the prevention of infection by *C. sakazakii* in foods such as infant formula. **Jahani et al**. [40] reported that lactoferrin had an antimicrobial effect on Gram-positive and Gram-negative bacteria.

Conclusion

Pantoea spp. should be considered one of the pathogens responsible for neonatal sepsis, which necessitates implementing infection control strategies. Also, PIF and PN may be considered the source of *Pantoea* spp. infection at the NICU. *Pantoea* spp. showed high resistance against imipenem, nalidixic acid, and ampicillin, whereas it was sensitive to gentamycin, tetracycline, chloramphenicol, and aztreonam. Anise oil, peppermint oil, hibiscus, and lactoferrin had an antimicrobial effect against *Pantoea* spp. in vitro. A large epidemiologic study is required to reinforce these results.

Conflict of interest

The authors report no conflicts of interest.

Financial disclosures

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References

1-**Kini K, Agnimonhan R, Dossa R, Silué D, Koebnik R.** A diagnostic multiplex PCR scheme for identification of plant-associated bacteria of the genus Pantoea. bioRxiv 2018; doi:10.1101/456806

2-**Mani S, Nair J.** Pantoea Infections in the Neonatal Intensive Care Unit. Cureus 2021; 13(2): e13103

3-**Maki DG, Rhame FS, Mackel DC, Bennett J V.** Nationwide epidemic of septicemia caused by contaminated intravenous products: I. Epidemiologic and clinical features. Am J Med 1976;60(4):471-485.

4-**Bergman KA, Arends JP, Schölvinck EH.** Pantoea agglomerans septicemia in three newborn infants. Pediatr Infect Dis J 2007;26(5):453-454.

5-**Aly NYA, Salmeen HN, Lila RAA, Nagaraja PA.** Pantoea agglomerans bloodstream infection in preterm neonates. Med Princ Pract 2008;17(6):500-503.

6-**Büyükcam A, Tuncer Ö, Gür D, Sancak B, Ceyhan M, Cengiz AB, et al.** Clinical and microbiological characteristics of Pantoea agglomerans infection in children. J Infect Public Health 2018;11(3):304-309.

7-**Völksch B, Thon S, Jacobsen ID, Gube M.** Polyphasic study of plant-and clinic-associated Pantoea agglomerans strains reveals

indistinguishable virulence potential. Infect Genet Evol 2009;9(6):1381-1391.

8-**Flores Popoca EO, Miranda García M, Romero Figueroa S, Mendoza Medellín A, Sandoval Trujillo H, Silva Rojas HV, et al.** Pantoea agglomerans in immunodeficient patients with different respiratory symptoms. Sci World J 2012.

9-**Dutkiewicz J, Mackiewicz B, Lemieszek MK, Golec M, Milanowski J.** Pantoea agglomerans: a mysterious bacterium of evil and good. Part IV. Beneficial effects. Ann Agric Environ Med 2016;23(2).

10-**Mardaneh J, Soltan Dallal MM.** Isolation, identification and antimicrobial susceptibility of Pantoea (Enterobacter) agglomerans isolated from consumed powdered infant formula milk (PIF) in NICU ward: First report from Iran. Iran J Microbiol 2013;5(3):263-267.

11-**Hamer D.** Clinical signs that predict severe illness in children under age 2 months: a multicentre study Young Infants Clinical Signs Study Group. Lancet 2008;371:135-142.

12-**Elkhawaga AA, Hetta HF, Osman NS, Hosni A, El-Mokhtar MA.** Emergence of Cronobacter sakazakii in Cases of Neonatal Sepsis in Upper Egypt: First Report in North Africa. Front Microbiol 2020;1-9.

13-**Habsah H, Zeehaida M, Van Rostenberghe H, Noraida R, Wan Pauzi WI, Fatimah I, et al.** An outbreak of Pantoea spp. in a neonatal intensive care unit secondary to contaminated parenteral nutrition. J Hosp Infect 2005;61(3):213-218.

14-**Standards P, Testing AS**. M100 Performance Standards for Antimicrobial.

15-**Hussein EM, Alsantali RI, Morad M, Obaid RJ, Altass HM, Sayqal A, et al.** Bioactive fluorenes. Part III: 2,7-dichloro-9H-fluorene-based thiazolidinone and azetidinone analogues as anticancer and antimicrobial against multidrug resistant strains agents. BMC Chem 2020;14(1):1-24.

16-**Khalifa RA, Nasser MS, Gomaa AA, Osman NM, Salem HM.** Resazurin Microtiter Assay Plate method for detection of susceptibility of multidrug resistant Mycobacterium tuberculosis to second-line anti-tuberculous drugs. Egypt J Chest Dis Tuberc 2013;62(2):241-247.

17-**Ginovyan M, Keryan A, Bazukyan I, Ghazaryan P, Trchounian A.** The large scale antibacterial, antifungal and anti-phage efficiency of Petamcin-A: new multicomponent preparation for skin diseases treatment. Ann Clin Microbiol Antimicrob 2015;14(1):1-7.

18-**Van Rostenberghe H, Noraida R, Wan Pauzi WI, Habsah H, Zeehaida M, Rosliza AR, et al**. The clinical picture of neonatal infection with Pantoea species. Jpn J Infect Dis 2006;59(2):120-121.

19-**Focà A, Puccio R, Matera G, Capicotto R, Liberto MC.** Sei casi di sepsi causata da pantoea spp. In un policlinico universitario calabrese. Microbiol Medica 2006;21(3).

20-**Bicudo EL, Macedo VO, Carrara MA, Castro FFS, Rage RI. Nosocomial** outbreak of Pantoea agglomerans in a pediatric urgent care center. Brazilian J Infect Dis 2007;11(2):281-284.

21-**Cruz AT, Cazacu AC, Allen CH.** Pantoea agglomerans, a plant pathogen causing human disease. J Clin Microbiol 2007;45(6):1989-1992.

22-**Boszczowski Í, Nóbrega De Almeida Júnior J, Peixoto De Miranda ÉJ, Pinheiro Freire M, Guimarães T, Chaves CE, et al.** Nosocomial outbreak of Pantoea agglomerans bacteraemia associated with contaminated anticoagulant citrate dextrose solution: New

name, old bug? J Hosp Infect 2012;80(3):255-258.

23-**Izzo I, Lania D, Castro A, Lanzini F, Bella D, Pagani A, et al.** Seven cases of port-a-cath contamination caused by Pantoea agglomerans in the Oncological Service of Iseo Hospital, Brescia (Italy). Le Infez Med 2014;22(2):152-155.

24-**Didier ME, Fischer S, Maki DG.** Total nutrient admixtures appear safer than lipid emulsion alone as regards microbial contamination: growth properties of microbial pathogens at room temperature. J Parenter Enter Nutr 1998;22(5):291-296.

25-**Adam HJ, Darweesh MF.** Prevelance and antibiotic susceptibility patterns of Pantoea spp. Isolated form clinical and environmental sources in Iraq. Int J ChemTech Res 2016;9(8):430-437.

26-**Cheng A, Liu CY, Tsai HY, Hsu MS, Yang CJ, Huang YT, et al.** Bacteremia caused by Pantoea agglomerans at a medical center in Taiwan, 2000--2010. J Microbiol Immunol Infect 2013;46(3):187-19427.

27-**Liberto MC, Matera G, Puccio R, Lo Russo T, Colosimo E, Focà E.** Six cases of sepsis caused by Pantoea agglomerans in a teaching hospital. New Microbiol 2009;32(1):119-123.

28-**Tiwari S, Beriha SS.** Pantoea species causing early onset neonatal sepsis: A case report. J Med Case Rep 2015;9(1):2-4.

29-**Piskernik S, Klančnik A, Riedel CT, Brøndsted L, Možina SS.** Reduction of Campylobacter jejuni by natural antimicrobials in chicken meat-related conditions. Food Control 2011;22(5):718-724.

30-**Topuz OK, Özvural EB, Zhao Q, Huang Q, Chikindas M, Gölükçü M**. Physical and antimicrobial properties of anise oil loaded nanoemulsions on the survival of foodborne pathogens. Food Chem 2016;203:117-123.

31-**Liang R, Xu S, Shoemaker CF, Li Y, Zhong F, Huang Q.** Physical and Antimicrobial Properties of Peppermint Oil Nanoemulsions 2012.

32-**Mancuso M.** The Antibacterial Activity of Mentha . Herbs and Spices 2020:1-10.

33-**Kosalec I, Pepeljnjak S, KUŠTRAK D.** Antifungal activity of fluid extract and essential oil from anise fruits (Pimpinella anisum L., Apiaceae). Acta Pharm 2005;55(4):377-385.

34-**Dorman HD, Deans SG.** Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol 2000;88(2):308-316.

35-**Xi C, Zhang Y, Marrs CF, Ye W, Simon C, Foxman B, et al.** Prevalence of antibiotic resistance in drinking water treatment and distribution systems. Appl Environ Microbiol 2009;75(17):5714-5718.

36-**Akarca G.** Industrial Crops & Products Composition and antibacterial e ff ect on food borne pathogens of Hibiscus surrattensis L . calyces essential oil. Ind Crop Prod 2019;137:285-289.

37-**Al-Alak SK, AL-Oqaili RMS, Mohammed BB, Abd-Alkhalik N.** Antibacterial activity of Hibiscus rosa-sinensis extract and synergistic effect with amoxicillin against some human pathogens. Am J Phytomed Clin Ther 2015;3(10):20-27.

38-**Arnold RR, Brewer M, Gauthier JJ.** Bactericidal activity of human lactoferrin: sensitivity of a variety of microorganisms. Infect Immun 1980;28(3):893-898.

39-**Hameed KGA.** Inhibitory Effect of Lactoferrin Against Cronobacter Sakasakii Isolated From Infant Formula Milk Powder. Assiut Vet Med J 2017;63(152):73-77.

40-**Jahani S, Shakiba A, Jahani L.** The Antimicrobial Effect of Lactoferrin on Gram-Negative and Gram-Positive Bacteria. Int J Infect 2015;2(3).

Hassan GM, Hassan EA, Agban MN, Refat NH, Hosni AM, Elkhawaga AA. Insights into the prevalence and the antimicrobial activity of natural product against *Pantoea* spp in neonatal intensive care unit. Microbes Infect Dis 2023; Article-In-Press, **DOI:** 10.21608/mid.2022.168260.1400.