The prevalence of Plesiomonasshigelloids among hospitalized and out-clinic diarrheal patients and the role of the aquarium as a source of infection

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ABSTRACT

Background: Plesiomonas shigelloids a new member of the Enterobacteriaceae family is regards an emerging enteric pathogen, it is a water and soil organism and has been isolated from fresh water fish and many animals, and most isolates from humans have from stool cultures of patients with diarrhea. Little is known about the prevalence of plesiomonas shigelloids infections in Salah Al-din city. Furthermore this study design identification scheme for clinical laboratory to identify plesiomonas shigelloids.

Study design and objectives: This study was performed to determine the prevalence of plesiomonas shigelloids in hospitalized (nosocomial) patients and out clinic patients that infected with diarrhea in Tikrit Teaching Hospital, and out clinic centers during the period from May 2012 to the end April 2013.

Also this study was performed the role of aquariums that spread in Salah Al-din city as a source of plesiomonas shigelloidsdiarrheal infection, by collecting water samples from these aquariums monthly in the same period of diarrheal cases collection.

Patients and water sampling: One hundred and ten diarrhea samples were collected, 24 samples from TikritTeaching Hospital as a nosocomial diarrheal infection and 86 samples were collected from out clinic centers in Tikrit and other cities. Also water samples were collected from 29 aquariums that diffuse in Salah Al-din cities as monthly.

Identification method: This study used simple and short identification scheme for clinical laboratory diagnosis, by culturing the samples in blood agar as a first culture and S.S agar as a selective culture, and used Grams stain, Hanging drop test, Oxidase test, Stirring test, and Inositol fermentation test as a microscopic and biochemical identification tests.

Results: generally plesiomonasshigelloidsdiarrheal infection revealed the prevalence of this bacteria of the total examined stool samples, from 110 diarrheal cases 44(40%) cases with P. shigelloidesand 66(60%) cases with other bacteria, 7(29%) nosocomial diarrheal infection, were regarded with P. shigelloidesinfection, and37(43%) from out clinic diarrheal infection, were infected with P. shigelloides, these positive results were identified in the end of spring and summer months more than other seasons. Also from 29 aquarium water samples were collected monthly from each aquarium, showed variant results from month to another, but most of the aquarium water samples were positive in spring and summer months. The patient's results showed that the adults were the most infected with P. shigelloidesthan children or infants. Investigation of antibiotic susceptibility was done for allplesiomonasshigelloidsisolates, the nosocomial diarrheal samples showed multidrug resistance to antibiotics than the out clinic and aquarium samples. Some of the virulence factors were determined in this bacterium, like it is ability to produce some enzymes (DNase, lipase and lecithenase, urease, gelatinase) and to produce hemolysin toxin, the virulence factored results showed that the all isolates were produce lipase and hemolysin only.

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Key words: ***P. shigelloides*****,hospitalized diarrheal infection, aquarium, diarrheal infection.**

Introduction

Theplesiomonads are Gram-negative; non-sporeformingstraight rods bacterium (0.8 to 1µm).They are also facultative anaerobes, being catalase and oxidasepositive. Carbohydrate is broken down with the production of acidonly. The plesiomonads are motile and have several polar flagella. The optimum temperature for growth is 30–37 □C, althoughplesiomonads will grow at a range of temperatures between 8 and44 □C. The pH range for growth is 5–7.7 and growth is inhibited by6% salt broth3.

These organisms were initially described by Fergusonand Henderson in 1947 and were known as C27. The C27 organismswere initially placed in the genus Pseudomonas as 'Pseudomonasshigelloides'. They were subsequently transferred to the genusAeromonasas 'A. shigelloides'. Habs and Shubert proposed the namePlesiomonasin 1962 and the organisms were given their own genus.The name for this genus was chosen from the Greek word for 'neighbor'as it was thought that the organism was closely related to Aeromonas.However, Plesiomonasspp.have been found to be more closely
to the genus Proteus in the family Enterobacteriaceae.
Plesiomonasshigelloidesis the only species in the genus. This speciesname was chosen, as a minority of strains share a common O-antigenwith Shigellasonnei. Despite this, the genus Plesiomonasstillresidesin the family Vibrionaceae1, 4.

The primary reservoir for this bacterium is aquatic environment.P. shigelloideshas been isolated from both freshwater (rivers, creeks, lakes, etc.) Andfrom seawater 5,6.Most of the reports on isolation of P. shigelloidesare from countries in tropicalor subtropical areas7. The high incidence of this bacterium in Japan,Thailand, and, more recently, China has given the acronym "Asian" to thismicro-organism. However, studies in Africa, among others, show that P. shigelloidesis globally distributed8,9. P. shigelloideshas been implicated as an agent of human gastroenteritis formore than a half century, and there are increasing numbers of reports describinginfections caused by this microrganism7. The most important vehicle fortransmission of P. shigelloidesto humans appears to be seafood 10, 11, thoughrecently transmission through contaminated vegetables was described12. The route of entry into the human gastrointestinal tract is throughthe ingestion of contaminated food or water.Most isolates from humans have from stool cultures of patients with diarrhea 2Symptoms associatedwithgastroenteritis caused by P. shigelloidesinclude diarrhea, vomiting, abdominalpain, and nausea, although chills, headache, and fever may also occur13.

Severalvirulence factors have been studied and described in the literature. They includeenterotoxins, adhesions, invasions, enzymes, and other products such astetrodotoxin and histamine that may be implicated in seafood poisoning 14. The role and contribution of these factors to the overall pathogenicpotential of this micro-organism are not fully elucidated yet15.Most clinical diagnostic laboratories concentrate on recovery of classicaletiological agents of gastroenteritis, such as Salmonella, Shigella, andEscherichia coli, and P. shigelloidesmay thus be overlooked in a routine examinationof stool samples16.

P. shigelloidesstrains are usually susceptible to second- and thirdgenerationcephalosporins, nalidixic acid, quinolones, co-trimoxazole,chloramphenicol and nitrofurantoin. Resistance to aminoglycosidesisvariable. Most P. shigelloidesstrains produce □-lactamase and aretherefore resistant to all penicillins; however these isolates aresusceptible to penicillins in combination with □-lactamase inhibitors17, 18.

Materials and Methods

This study was conducted in the period from May 2012 to the end of April 2013 in Salah Al-din city. One hundred and ten stool samples were collected from patients with diarrhea from Tikrit Teaching Hospital and out clinic centers, and twenty nine water samples were collected from twenty nine water aquariums monthly from May 2012 to April 2013, that diffused in Salah Al-din city.

The samples of study were collected according to standard microbiology laboratory techniques. Stool collection was achieved via sterile plastic containers provided with screw caps. The samples were processed within two hours of collection. Stools were processed and analyzed for enteric bacteria on the day of sample collection. Standard culture and identification methods were used to identify P.shigelloidesin the second day by using short laboratory identification scheme as shown in figure 1, you can used this scheme when identifying bacteria that encountered in clinical laboratories. Samples were cultured on Blood agar and Salmonella.Shigella agar. Cultured plates were incubated overnight at 35°C. After incubation the bacterial isolates were identified according to its morphological, and biochemical characteristics as recommended by 23, 37, 13.

Numbers of antibiotics for sensitivity test were determined by Kirby Bauer disk diffusion method, and some of virulence factors were investigated for all P. shigelloides isolates as recommended by 19-20.

For aquarium water samples, the Plate Count Techniques were useful for enumerating the naturally occurring bacteria that exist in a particular environment 21. This method detects spatial and seasonal variations in microbial populations and can serve as a useful source of isolates for further study.

Results and Discussion

Isolation and Identification:

One hundred and ten diarrhea samples were collected, 24(22%) samples from Tikrit Teaching Hospital as a nosocomial diarrheal infection and 86(78%) samples were collected from out clinic centers in Salah-Al-din cities. Plesiomonasshigelloideswere detected in 44(40%) cases and 66(60%) cases were negative for P. shigelloides, as shown in figure (2).

Also, the seasonal variation of positive P. shigelloides cases showed that these cases were detected in May and June more than other months for nosocomial diarrheal infection and the out-clinic cases, as shown in figures (4, 5).

Plesiomonasshigelloidshas been isolated from stool samples of patients with diarrhea 24. The present cross sectional study focused on the role and prevalence of these bacteria first as a cause of nosocomial diarrheal infections and second as out clinic diarrheal infections. No clear results mentioned the role of P.shigelloidesin nosocomial diarrheal infection but 22, 30 mentioned that these bacteria can cause nosocomial diarrheal infections in hospitalized patients with weak immune system.

Also many reasons leads to nosocomial diarrheal infection, the hands of health care workers provide the major source of transmission of pathogens from patient to patient25, also oral ingestions seems to be the major route of introduction of nosocomial pathogens into the intestinal tract 26, and we know that most cases of P. shigelloidesdiarrheal infections result from eating foods contaminated with large number of these bacteria 22.

Once over growth of nosocomial pathogens has been established in the colon, fecal contamination results in spread to the environment and patient's skin, the density of colonization by pathogens may have important implications for transmission to digestive system27.

For the more, the inhibiting of colonization resistance and overgrowth of potential pathogens by antibiotics treatments that are excreted into intestinal tract is the most important factor that may disturb colonization resistance of patients 28. Finally contamination may be related to variety of factors that result in reduced standards of hygiene among hospitalizedpatients (e.g. sever illness, alteration of mental status, and debilitation) 29.

For out-clinic patients current results are more than mentioned by many researchers19, 20and 31, they isolated P. shigelloidesfrom ambulatory patients in 16.5%, 3.3% and 1.3% respectively. Infections caused by P. shigelloidesoccur more commonly among low-income groups in poorer areas32.Also several factors can contribute to P. shigelloidesdiarrheal infection, the duration of maternal breastfeeding, food and environmental contaminations, maternal education and employment, home location, family income, access to treated water and basic sanitary conditions, seasonal variations and hosts underlying conditions 33, 34. Furthermore P.shigelloidesisolated from a wide range of different animals, such as freshwater fish, goats, swine, cats, dogs, and also from fresh vegetables, that may play an important role in P.shigelloidesdiarrheal infections1.

Our study was carried out on the end of spring and summer, the most diarrheic season in Iraq, and because Iraq is one of the subtropical climate countries where the P. shigelloidesmay present year around 7.

Twenty nine aquariums were selected for this study, to detect the prevalence of P. shigelloidesin water samples that collected from these aquariums that diffused in Salah-Al-din city. The water samples were collected monthly from each aquarium. Plate count technique was doing for each water sample. As shown in figure (6) almost aquarium samples were positive for P. shigelloidesin the end of spring months and summer months than for autumn and winter months.

The second targets of our study prove the role of aquariums that diffused in Salah Al-din cities in P. shigelloidesdiarrheal infection. We notice the positive results in water samples from these aquariums especially in summer months, whichare similar with the patient's results, as seen with previous figures.Also, most people who ate fishes taken from this aquarium in restaurants, especially in the spring and summer monthswere suffering from diarrhea that may be due to P.shigelloides infection. Plesiomonasshigelloidesisfound inaquatic environments primarily freshwater and estuaries within tropical and temperate climates in summer months 22.

Age distribution:

As shown in table (1) the most age that infected by P. shigelloideswas adults than children, and no positive cases with infant.These results confirmed by 13, he mentioned that most cases of P. shigelloidesdiarrheal infection were adults.

Numbers of virulence factors were investigated for all P. shigelloidesisolates as shown in table (2). All isolates were produced hemolysin toxin, andlipase, whereas all isolates do not produced gelatinase,DNase,lecithenase, and urease.

These results were in agreement or closed to those reported by other study 35they determined the ability of P. shigelloidesto produce hemolysin toxin, and lipase. Hemolysin toxin can utilize the iron found in hemoglobin. Whereas the results of urease and gelatinase production were agreements with 19, 20, 38and 39, they referred that P. shigelloidesisolates cannot produce Urease, lecithenase,Gelatinase, andDNase. P. shigelloidesappears to possess a much lower virulence potential with low symptomatic carriage rate among humans 13.

The standard disk diffusion method was used to determine the sensitivity of P. shigelloidesisolates to several antibiotics, as shown in Table (3). This table showed that all isolates were resistance toAmikacin, Ampicillin, Carbenicillin, Cefotaxime,Gentamicin, Rifampicin and Streptomycin. Whereas, sensitive for, Chloramphenicol, Ciprofloxacin, Trimethoprim, andNalidixic acid. For Azithromycin, tetracycline, and amoxicillin-clavulunic acid the nosocomial and ambulatory isolates were resistance, whereas sensitive for aquarium isolates.

These results were close to those reported by other studies, which found that most strains of these bacterium were multiresistant20. This result showed that the clinical isolates were multiresistant to antibiotics more than the aquarium isolates, and this result is in agreement with 20.Antimicrobial resistance in enteric pathogens is of great importance in developing world, were the rate of diarrheal diseases is highest 31. Studies have shown a general resistance to the penicillin class of antibiotics, but trimethoprim are active. There are reports of resistance to more than one aminoglycoside (e.g. gentamicin, and amikacin) 13, 36. Hospitalized patients carrying these resistant isolates may continue to carry them over prolonged periods, and continued carriage of such isolates might contribute to their propagation both within and outside of hospital environment 30.Plesiomonasshigelloidesare usually susceptible to nalidixic acid, quinolones, chloramphenicol, and trimethoprim, whereas resistance to streptomycin, and rifampicin 18. Treatment of P. shigelloidesassociated infection should be guided by sensitivity testing of the isolate. Quinolones may be used to treat P. shigelloidesassociated gastroenteritis and in some cases antibiotic treatment has been shown to decrease the duration of diarrheal illness 18.

Conclusions

The results of this study emphasize the importance of P. shigelloidesbacteria in causing infectious diarrhea in hospital or outpatient clinics. Hence, it is advisable to be diagnosed routinely in all public and private hospital labs when other more common causes were excluded. Malnutrition factor has shown to be main independent risk factor for developing infection.

Acknowledgment: I acknowledge the support and assistance of Dr. SinanBahjatAlrifai, and all the doctors, nurses, and laboratory staff of Tikrit Teaching Hospital, and out- clinic centers, also for the aquarium farmers,for their assistance and cooperation.

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TABLES

Table (1) Age distribution for *P. shigelloides*diarrheal infection.

Age	Nosocomial cases		Out-clinic cases	
	+ve	-ve	+ve	-ve
Infant (< 1 yr)	0	5	0	2
Children (1-14 yr)	2	6	15	17
Adult (> 15 yr)	5	6	22	30
Total	7	17	37	49

Table (2): Virulence factors for *P. shigelloides*isolates.

Isolate	Hemolysin	Lipase	Lecithenase	Urease	Gelatinase	DNase
P. shigelloides isolates	β	+	-	-	-	-

β :beta(complete hemolysis), (-): absence,(+):presence. N: nosocomial, C: out-clinic, W: water.

Table (3): Antibacterial resistance of P. *shigelloides*isolates.

Isolate	AK	AMP	PY	CTX	C	CF	GM	TR	TE	NA	RiF	AZM	AMV	S
N1-N7	R	R	R	R	S	S	R	S	R	S	R	R	R	R
C1-C37	R	R	R	R	S	S	R	S	R	S	R	R	R	R
W1-W28	R	R	R	R	S	S	R	S	S	S	R	S	S	R

R: Resistance, S:Sensitive. N: nosocomial, C: out-clinic, W: water.AK:Amikacin, AMP:Ampicillin, PY: Carbencillin, CTX: Cefotaxime, C: Chloramphenicol, CF: Ciprofloxacin, GM: Gentamicin, TR: Trimethoprim, TE: Tetracycline, NA: Nalidixic acid, Rif: Rifampicine, AZM: Azithromycin, AMV: Amoxycilline-clavulonic acid, S:Streptomycin.

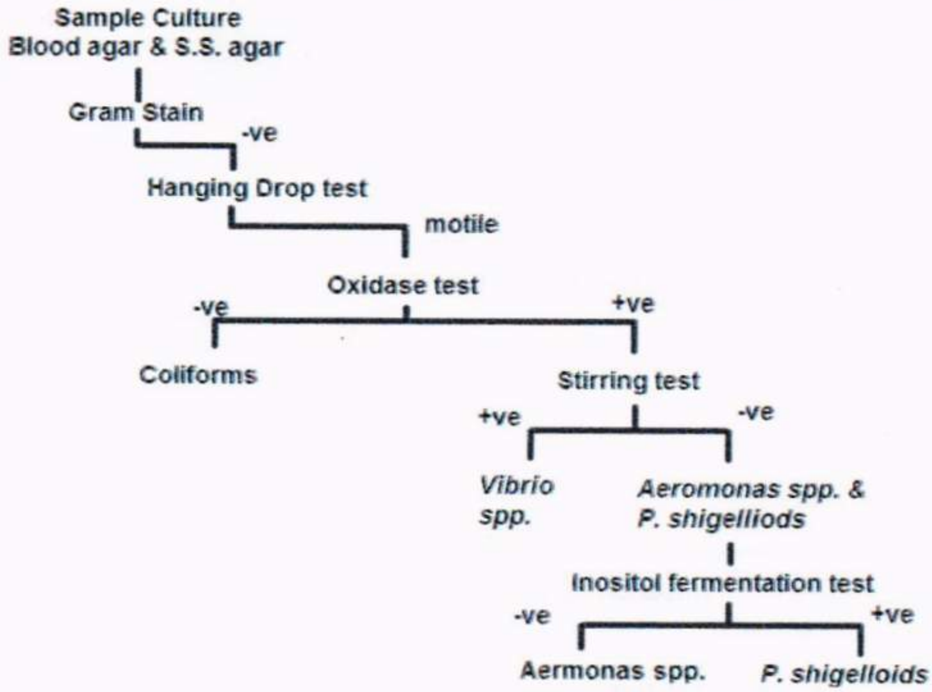

Figure (1) Laboratory identification scheme for P. *shigelloides* .

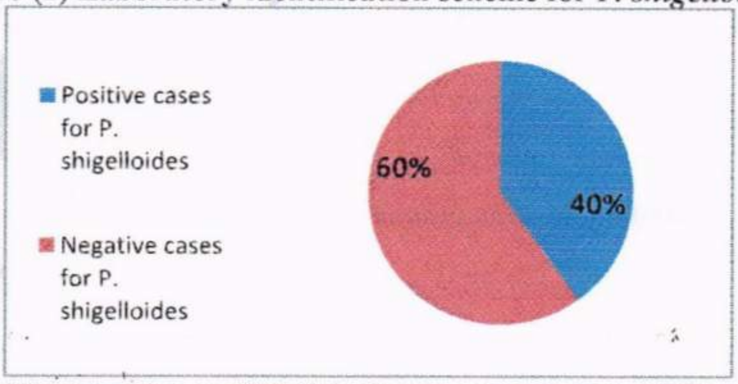

Figure (2): The prevalence of P. shigelloiges among current diarrhea cases.

For nosocomial cases, 7(29%) were *P.shigelloides*and for out-cliniccases, 37(43%) were *P.shigelloides*as shown in figure (3).

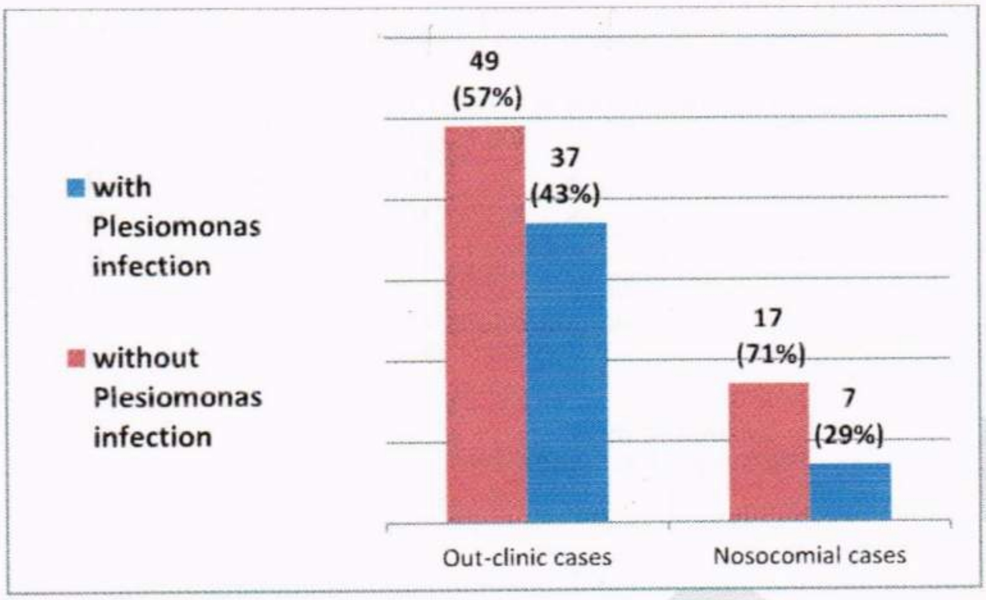

Figure (3): The distribution of positive and negative cases among both out-clinic and nosocomial cases.

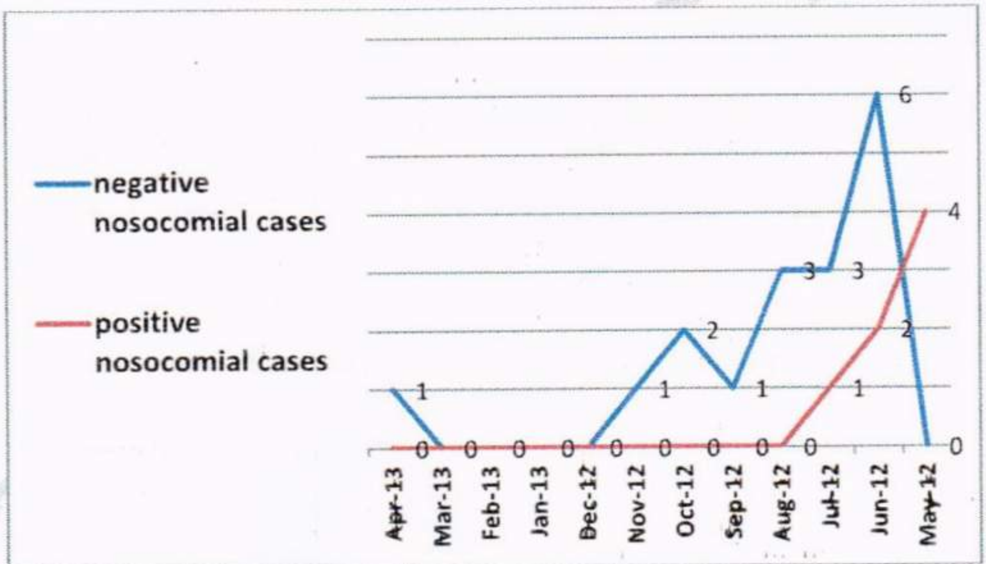

Figure (4): The distribution according to seasonal variation for positive nosocomial cases.

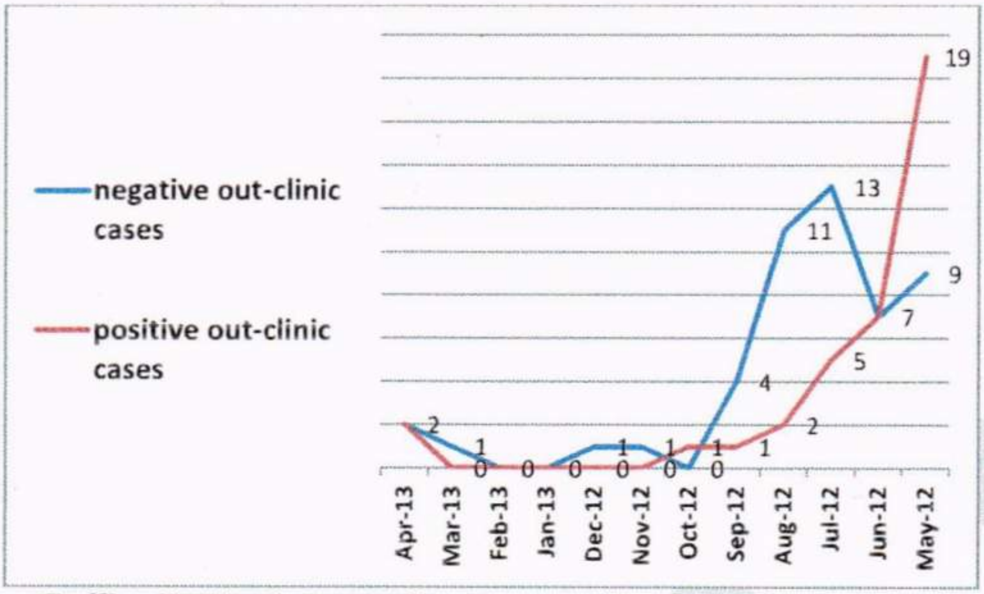

Figure (5): The distribution according to seasonal variation for positive out-clinic cases.

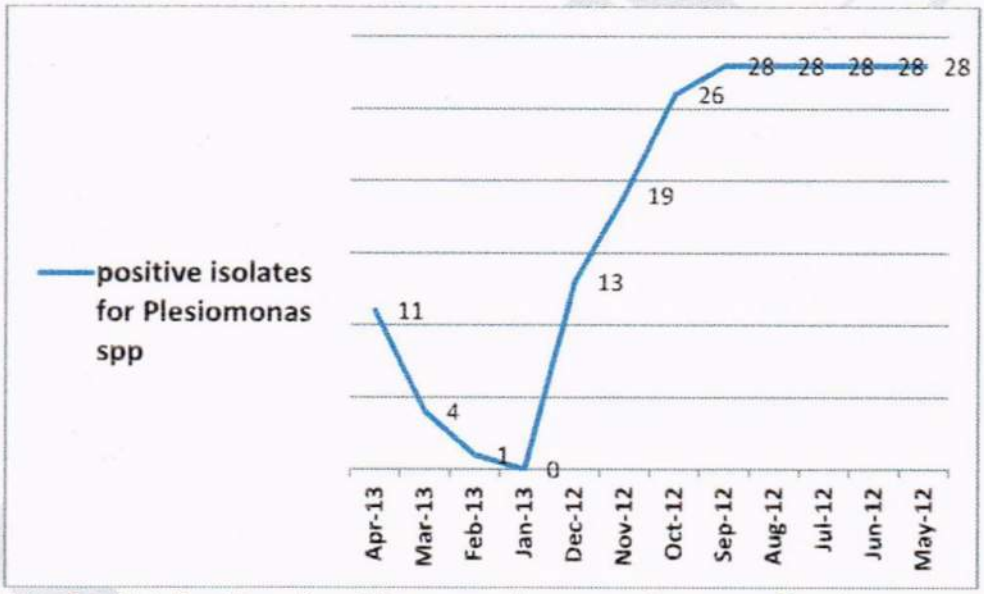

Figure (6): the distribution of positive aquarium samples according to seasons.