TMKARPIŃSKI
PUBLISHER

ISSN 2300-7257

Volume 2
Number 2
July-December 2014

Archives of Biomedical Sciences

http://www.journals.tmkarpinski.com/index.php/abms
e-mail: abms@interia.eu

Archives of Biomedical Sciences
ISSN 2300-7257

Publisher and Editor's office:
Tomasz M. Karpiński, Szkółkarska 88B, 62-002 Suchy Las, Poland, e-mail: ambs@interia.eu
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Contents

TMKARPIŃSKI PUBLISHER

Archives of Biomedical Sciences

ISSN: 2300-7257

ORIGINAL ARTICLE

Prevalence of some pathogenic bacteria of raw milk in Algeria

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Received: 04 June 2014; **Revised submission:** 03 July 2014; **Accepted:** 04 July 2014

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ABSTRACT

This study was conducted in order to assess the microbial load of raw milk produced in Algeria by some toxic bacteria. In total, 30 samples were collected for 6 weeks from the point of delivery of the state dairy of Boumerdes in 5 tanks of different collectors. The analysis results showed that 13% of milks were contaminated with *Listeria.* 7 samples were positive for *Staphylococcus aureus*, with an average count expressed in Log10 cfu/ml of 1,85 ± 0,68. Positive coagulase were 79% of *S. aureus* strains. The average value of *E. coli* bacteria was considerable: 5,64 ± 1,27. The sulphite-reducing *Clostridia* detected in 9 samples were achieved varying levels between 0 and 60 cfu/ml. All samples were free of *Salmonella* spp. Significant differences in the profiles of various studied microbial pathogens indicate a deterioration in the microbiological quality of raw milk.

Key words: Raw milk; Collection; Pathogens; Bacteria; Algeria.

1. INTRODUCTION

Milk is a food with high nutritional value but its physico-chemical properties make it a very favorable medium to the growth of microorganisms [1]. The main risk to fear is contamination by pathogenic germs. Beyond the direct impact on human health, the contaminated milk is an economic barrier for the dairy industry. The origin of the contamination by these pathogens varies depending on the nature of the product and conditions of its production and processing. This contamination can be of endogenous origin and is then due to udder excretion of sick animal. It may also be of exogenous origin, then it is a direct contact with infected herds or contribution of the environment (water, staff, etc.) [2]. This study aims to assess the microbiological quality of raw milk produced in two regions of Algeria (Algiers and Boumerdes) through the detection and enumeration of five pathogenic bacteria relevant to the dairy industry and considered toxic [3], namely: *Escherichia coli*, *Clostridia*, *Salmonella* spp., *Staphylococcus aureus* and *Listeria monocytogenes*.

2. MATERIAL AND METHODS

2.1. Material

A total of 30 raw milk samples were aseptically collected and analyzed on a weekly basis for 6 weeks in spring season, from 5 tanks of different collectors delivering milk to the dairy state of Boumerdes.

2.2. Methods

For each sample, the decimal dilutions at 10^{-6} were realized.

- *Escherichia coli* were enumerated on deoxycholate at 1‰ agar. The positive result is the appearance of round, red colonies.
- *Staphylococcus aureus* were performed on Baird Parker agar supplemented with egg yolk and potassium tellurite. The positive test result is the appearance of colonies surrounded by a bright yellow halo with a black center. They were then picked and tested for catalase and coagulase.
- The *Clostridia* were enumerated on the meat liver agar added to iron alum and sodium sulfite. Only black colonies were counted.
- For *Salmonella* spp. a pre-enrichment on lactose "mannitol" buffered broth medium was followed by an enrichment on sodium selenite and cysteine broth (SFB). Enumeration and isolation were performed on Hektoen agar. *Salmonella* colonies appear with a greenish blue color and a black center.
- *Listeria monocytogenes*: it requires prior enrichment in Fraser ½ broth in first day. The second day, an enrichment in Fraser broth was made. Isolation is in the 3rd day on Palcam agar. The positive result is manifested by the appearance of greenish colonies.

2.3. Statistical analysis

Microbiological results were transformed into Log10cfu/ml. Data were analyzed with the following modules of the STATISTICA 8.0. The significance level was fixed at $p < 0.05$.

3. RESULTS AND DISCUSSION

Pathogenic bacteria searched then enumerated in the present study were taken in photos in their respective culture media as showed in Figure 1.

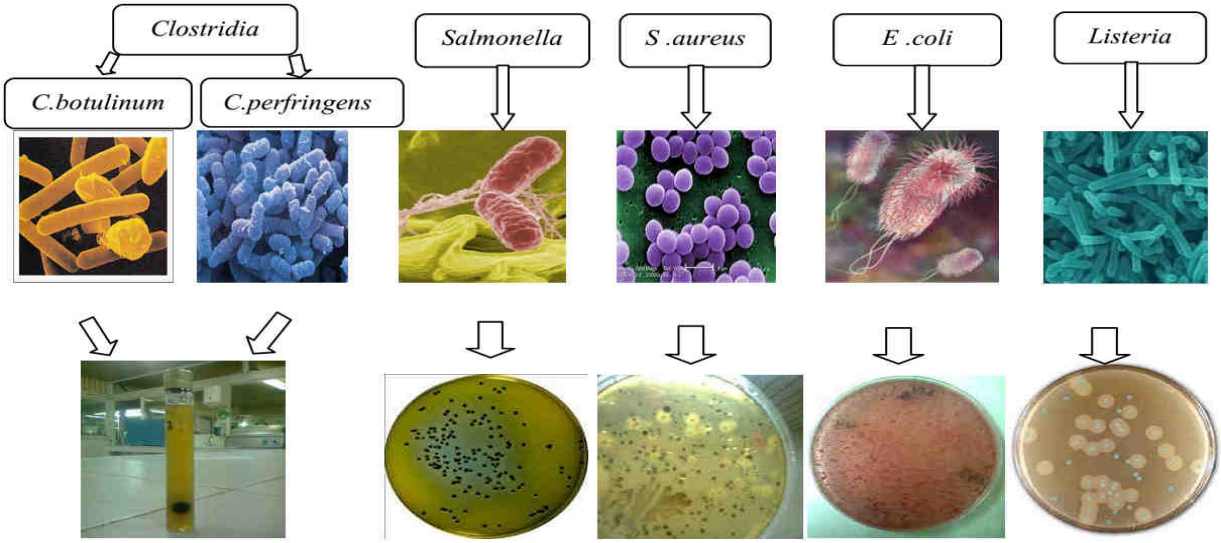

Figure 1. Aspects of different pathogens in their culture media.

Microbiological analyzes for the detection of *Staphylococcus aureus* and *Escherichia coli* showed varying levels (Figure 2). Search in the raw milk of *Staphylococcus aureus* revealed its absence in nearly 77 % of the samples (n= 23). This germ is considered the main cause of clinical and subclinical mastitis in dairy cattle farms [4]. The average charge per milliliter was $7{,}2\times10^{1}$ cfu. Values were variable from 0 to 5×10^{2} cfu/ ml. The presence was detected in four samples of the third collector's tank with an average of 2,47 Log10 cfu/ml. Tanks of the 2nd and 5th collector were completely unharmed. These results were significantly lower compared to those found in cote d'Ivoire [5], in western Algeria [6], in the region of Tiaret in Algeria [7] and in Morocco [8], where a very large samples contamination by *S. aureus* has been reported reaching averages in cfu/ml respectively: $2{,}1\times10^{3}$, 35×10^{2}, 2×10^{3} and $4{,}6\times10^{3}$. However, contamination by *S. aureus* at a rate of 60 % for an average count of 12×10^{3} cfu/ml has been reported [9]. The catalase test conducted

showed that most colonies were positive with average rates that fluctuate between 83.5% and 100%. In parallel, 79 % of the colonies tested were positive coagulase.

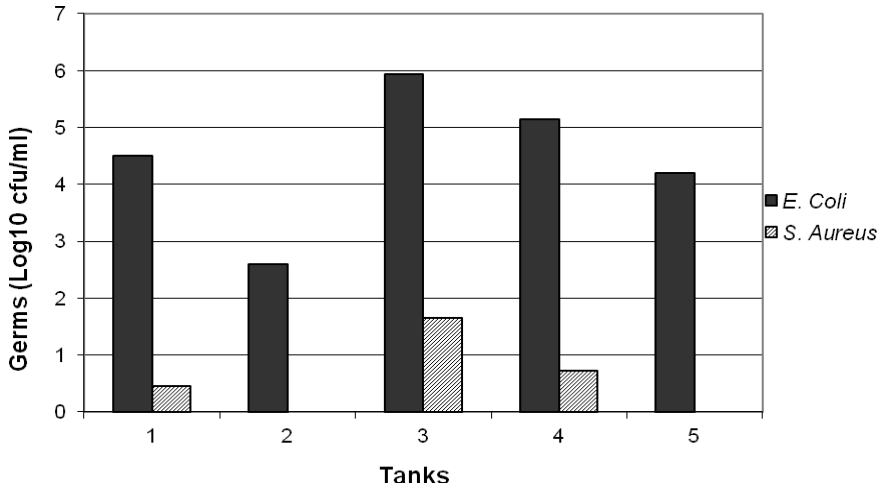

Figure 2. Contamination rates by *Staphylococcus aureus* and *Escherichia coli* of the samples analyzed.

About *Echerichia coli*, approximately, 94% of the samples were contaminated by this germ with an important average count of 5,64 ± 1,27 Log10 cfu/ml (45×10^4 cfu/ml), and average values between 0 and 6,74 Log10 cfu/ ml. The second tank shined with 2 totally free samples during the 3rd and 5th week of our study and the lowest average count estimated at 2,6 ± 2,04 Log10 cfu/ml. The highest average (5,94 ± 0,3 Log10 cfu/ml) was recorded in the third tank. Our results were slightly higher compared to $5,5\times10^2$ cfu/ml [5] and $2,1\times10^3$ cfu/ml [8]. While, in Mali high values in the range of 8×10^6 cfu/ml were reported [10]. These bacteria indicate a fecal contamination and testify deteriorated sanitary conditions during milking or/and during transport even if they are present at low levels in milk. The *sulphite-reducing Clostridia* detected in 30% of samples have achieved varying levels from 0 to 60 spores/ml. The average contamination of all milks was nearly 5 spores/ml. The second tank was totally free from this germ. Low average of about 1 to 12 spores/ ml were recorded in the positive samples detected in three tanks (1st, 4th and 5th). The highest rate was found during the first week at the 3rd collector reached 60 cfu/ml. This microbial group is on average very low in milk. In France, about 180 spores/l were found [11]. While, Aggad et al. [6] reported rates between 20 and 29 germs/ml. In contrast, 13% of milks (n=4) were contaminated with *Listeria*. The average count per milliliter was about 1 spore. However, few studies have been conducted to estimate the frequency of this pathogenic bacteria in raw milk cattle in Algeria. The only studies, one conducted [12] found that among 153 milk samples which were collected from farms in the regions of Algiers and Blida, 2,61% were contaminated. In the other study they found a rate of contamination of 5,76% [13]. However, microbiological testing for *Salmonella spp* showed no contamination in all samples tested, which may indicate a good state of health of the cows. Through these results, it was found that the tank 3 was characterized by a large number of contaminated samples with the highest averages count of pathogens. This seems to be closely related to the large number of farms collected ($p<0,05$). Often healthy milk were contaminated after being mixed with the contaminated milk from other farms where the multiplication and the spread of germs existing.

4. CONCLUSION

A large variability was observed in the number and type of the detected microorganisms, indicating a defect in the microbiological quality of raw milk. It was concluded that this contamination of raw milk by these pathogens is linked to the mixture of milks of several farms, as well as non-compliance with hygiene during milking and transport. In the future, the establishment of standards for good hygienic practices at all links in

the chain will prove crucial to the development of the dairy sector in Algeria.

ACKNOWLEDGEMENTS

The authors would like to thank all the staff of the laboratory quality control of dairy unit of Boudouaou (Boumerdes, Algeria)

AUTHORS' CONTRIBUTION

All authors contributed effectively to the data collection, analysis and interpretation of results, drafting and revision of the manuscript.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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TMKARPIŃSKI
PUBLISHER

Archives
of Biomedical
Sciences

ISSN: 2300-7257

ORIGINAL ARTICLE

Antimicrobial activity of crude extracts of cyanobacteria *Nostoc commune* and *Spirulina platensis*

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Received: 11 July 2014; **Revised submission:** 31 August 2014; **Accepted:** 16 September 2014

www.journals.tmkarpinski.com/index.php/abms

ABSTRACT

Cyanobacteria inhabit a range of diverse and extreme habitats and have potential to produce an elaborate array of secondary metabolites with unusual structures and potent bioactivity. Libya is well known as an area of high biological diversity. In our study, fifteen cyanobacteria from the natural area were isolated and screened for their antimicrobial activities. Cyanobacteria were extracted in water and ethanol, and tested for antimicrobial activity against seven bacteria (*Serratia*, *Escherichia*, *Bacillus*, *Micrococcus*, *Staphylococcus*, *Klebsiella* and *Pseudomonas*) and *Aspergillus flavus* for antifungal activity. Aqueous and ethanol extracts of the blue green alga *Anabaena circinalis* exhibited antibacterial activity against *Serratia marcescens* and *Escherichia coli*, however it has activity against *Klebsiella pneumoniae* and the fungus *Aspergillus flavus* using only ethanol extracts. Also, the *Nostoc commune* exhibited significant activity against *E. coli*, *S. marcescens* and *Bacillus cereus* in addition to *K. pneumoniae* and *Micrococcus luteus*. The other blue green alga *Nostoc muscorum* has wide range activity on bacteria Gram-positive bacteria (*Staphylococcus aureus, M. luteus* and *B. cereus*) and Gram-negative bacteria (*Pseudomonas aeruginosa*, *K. pneumoniae* and *S. marcescens*) in addition to the fungus *A. flavus*. As regards the dominant species of cyanobacteria *Spirulina platensis* under investigation, the aqueous extract of *Spirulina platensis* has antibacterial activity against all species tested except *B. cereus* and *P. aeruginosa*. They exhibited significant activity against *S. aureus, E. coli, S. marcescens, B. cereus, K. pneumoniae* and *M. luteus*, in addition to the fungus *A. flavus*. Therefore, two cyanobacteria may be useful in various applications and used as basic knowledge for further investigations.

Key words: Cyanobacteria; Bacteria; Fungi; Antibacterial activity.

1. INTRODUCTION

Cyanobacteria are an incredibly old group of prokaryotic organisms that produce a variety of industrially important secondary metabolites such as antibiotic, algicide, cytotoxic, immunosuppressive and enzyme inhibiting agents. Cyanobacteria are a morphologically diverse group of Gram-negative eubacteria. It is able to perform oxygenic photosynthesis and used as important food for other organisms. Moreover, it is widely found in various locations such as pond, soil, rock, bark, sea and fresh water [1]. Cyanobacteria are several potential benefits to study on bioactive compounds from these organisms. Although, antibacterial, antiviral, algaecide, antifungal and cytotoxic activities have

been much researched in these organisms [2-5]. Cyanobacteria are one of the most promising groups of organisms for isolation of novel and biochemically active natural products [6, 7]. A number of research papers have been published recently about the antimicrobial activities from cyanobacteria [5, 8-12]. The cyanobacterium *Lyngbya majuscula* is responsible for sporadic outbreaks of a contact dermatitis known as 'swimmer itch'. The cyanobacteria such as *Nostoc commune* [13, 14], *Anabaena variabilis* [15], *Nostoc spongiaeforme* [16], *Microcystis aeruginosa*, *Anabaena flos-aquae* [17], *Trichodesmium erythraeum* [18], *Nodularia harveyana* [4] and *Calothrix brevissima* [19] have been popularly reported to produce antimicrobial substances. Heptadecane and tetradecane from *Spirulina platensis* [20], phenolic compounds from *Nostoc muscorum* [21], peptides, polypeptides, amides and alkaloids from *Fischerella ambigua* [22], lipopeptidases from *Anabaena* spp. [7, 23], fatty acids, tetramine, spermine and piperazine derivative from *Anabaena* spp. [24, 25], laxaphycins from *Anabaena laxa* [26] and scytophytin from *Scytonema psuedohofmanni* [27] have been reported to possess antimicrobial activity. In order to explore cyanobacteria with medical potentials, cyanobacteria isolated from Libyan soil were screened antimicrobial activity against seven bacteria, and one fungus. Most of the cyanobacteria species were new and information about antimicrobial activity very limited.

2. MATERIAL AND METHODS

Soil samples were cultured by usual methods [27]. Cyanobacteria were grown in 250 ml conical flasks containing 100 ml of ASN-III medium adjusted to pH 7.4. The cultures were grown at 25 ± 2°C and illuminated (50 µmol photons m^{-2} s^{-1}) under cool fluorescent lights of 12:12 L:D cycle. The culture media were bubbled with 0.3% CO_2-enriched air. Standard plating and streaking techniques were used for isolation and purification of cyanobacteria [29]. Identification of cyanobacteria with antimicrobial activity was done according to Desikachary [30], Prescott [31], Anagnostidis and Komarek [32], and John et. al. [33]. Cyanobacterial biomass were harvested in the stationary growth phase by centrifugation at 5000×g for 15 min. 1 g of dried biomass of the isolates was extracted with ethanol in a mortar pestle and kept overnight at 4°C for complete extraction. The supernatant was collected after the centrifugation at 10000×g at 10 min. The solvent extracts were concentrated under reduced pressure at 40°C. Dry residue was re dissolved in dimethylsulfoxide (DMSO) and kept at 4°C until use for bioassay. The antibacterial activities of cyanobacterial extracts were evaluated by agar plate diffusion test (*E. coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus cereus*) and one fungus (*Aspergillus flavus*). Filter paper disks (5 mm) were saturated with 20 µl of 1 mg ml^{-1} test solution, dried, and placed on nutrient agar plates with a lawn of the test microorganisms. Plates were incubated at 37°C and inhibition zones were measured. The growth rate and generation time of a test alga grown with various N concentrations were followed by daily measurements of absorbance at 750 nm. Optical density was used as a parameter for algal growth. After 10 days growth in case of *Spirulina platensis*, and 10 days in case of *Nostoc commune* the algal cells were harvested for some metabolic estimations, in the late of exponential phase or beginning of the stationary phase according to algal growth curve as shown later [34]. Chlorophyll-a was extracted in acetone (90%) and determined according to Marker [35].

3. RESULTS AND DISCUSSION

In vitro antibacterial activity of aqueous and organic extracts, each of fifteen cyanobacterial species were evaluated against Gram-positive bacteria (*Staphylococcus aureus*, *Micrococcus luteus* and *Bacillus cereus*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Klebsiella pneumoniae*). The fifteen dominant species tested for antimicrobial activity are listed in Table 1. Aqueous and ethanol extracts of the blue green alga *Anabaena circinalis* exhibited antibacterial activity against *S. marcescens* and *E. coli*, however it has activity against *K. pneumoniae* and the fungus *Aspergillus flavus* using only ethanol extracts. Also, the *Nostoc commune* exhibited significant activity against

E. coli, *S. marcescens* and *B. cereus* in addition to *K. pneumoniae* and *M. luteus*. The other blue green alga *Nostoc muscorum* has a wide range activity on bacteria e.g Gram-positive bacteria (*S. aureus*, *M. luteus* and *B. cereus*) and Gram-negative bacteria (*P. aeruginosa*, *K. pneumoniae* and *S. marcescens*) in addition to the fungus *A. flavus* (Table 1).

Table 1. The antimicrobial activity of some cyanobacterial species against bacteria and fungi throughout the study periods.

Cyanobacteria	*Serratia marcescens*		*E. coli*		*Bacillus cereus*		*Micrococcus luteus*		*Staphylococcus aureus*		*Klebsiella pneumoniae*		*Pseudomonas aeruginosa*		*Aspergilus fluvas*	
	Solvent		Solvent		Solvent		Solvent		Solvent		Solvent		Solvent		Solvent	
	W	E	W	E	W	E	W	E	W	E	W	E	W	E	W	E
Anabaena circinalis	+	+	++	+	--	--	-	-	--	--	--	+	--	--	--	+
Chroococcus minor	++	+	+	--	--	--	--	--	--	--	--	--	--	--	+	--
Lyngbya sp.	--	--	+	++	+	+	+	+	+	+	--	--	+	+	--	--
Lyngbya contorta	+	+	++	--	--	--	+	+	+	--	--	--	--	+	--	--
Merismopidia sp.	+	+	--	+	--	--	--	--	--	--	--	--	+	--	--	--
Microcystis sp.	+	+	+	++	+	+	+	--	--	+	--	--	+	+	--	--
Nostoc commune	+	+	+	+	+	+	+	+	--	--	--	+	--	--	+	--
Nostoc linkia	+	+	--	+	--	--	+	--	+	--	+	+	--	--	--	--
Nostoc muscorum	++	++	++	++	+	+	+	+	+	+	+	--	+	+	+	--
Oscillatoria formosa	++	+	++	+	+	--	--	--	+	+	--	+	--	--	--	--
Spiurilina platensis	+	+	+	++	--	--	+	--	--	++	--	--	+	+	+	--
Chroococcus turgidus	+	+	+	+	--	--	--	--	+	+	+	+	--	++	--	--
Gelocapsa sp.	+	+	+	+	+	--	+	--	--	--	+	+	+	+	--	--
Phormidium molle	--	--	--	--	--	--	+	+	--	--	--	+	--	--	--	+
Woella saccata	--	--	+	--	--	--	+	+	+	--	+	+	--	--	+	+

(W= water, E= Ethanol, + = zone 0.9<, ++ =zone >0.9, - = no activity.)

The effect of nitrogen concentrations (deprived nitrogen (–N), control (C), double nitrogen in medium (+2N), triple nitrogen (+3N) and fourth (+4N) on the growth curve of *Nostoc commune* was illustrated in Fig. 1, it was enhanced by all nitrogen applied (except –N). The highest enhancement effect was exerted by +3N and +4N. The maximum growth rate (0.221 $\mu.d^{-1}$) as well as minimum generation time (23.9 $G.d^{-1}$) for *Nostoc* was recorded in culture supplemented with +4N. Chlorophyll a and the dry mass were markedly increased with increasing nitrogen concentration (Table 2).

The data in Table 2 show that, there is no obvious trend between the nitrogen concentrations used in this investigation and the production of antimicrobial activity of *N. commune*. Aqueous and ethanol extracts of *Nostoc* in the nitrogen deprived medium (-N) has wide range of antibacterial in comparison to control and nitrogen supplemented cultures.

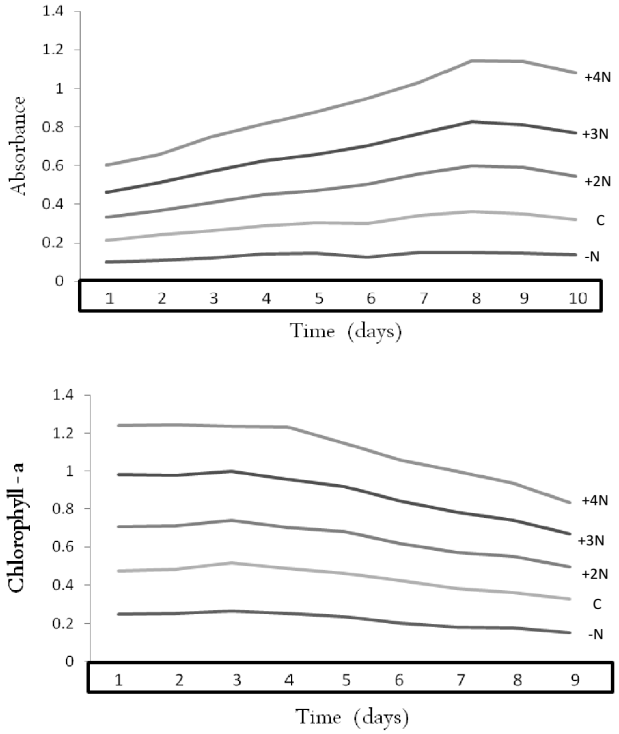

Figure 1. Growth curve of *Nostoc commune* under various nitrogen concentrations. Values are means of three replicates ± S.E. is smaller than the symbol in all cases.

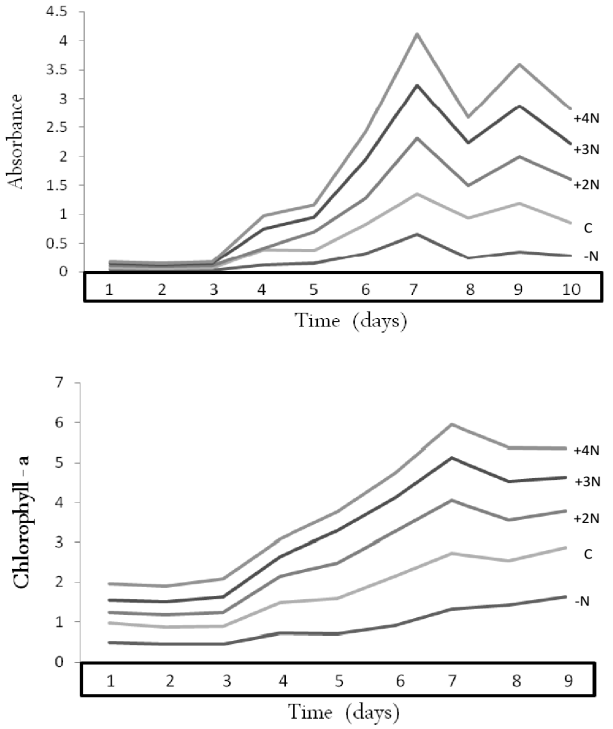

Figure 2. Growth curve of *Spirulina plataensis* under various nitrogen concentrations. Values are means of three replicates ± S.E. is smaller than the symbol in all cases.

Table 2. Effect of various nitrogen concentrations on the production of antibacterial by *Nostoc commune.*

Treatment	*Serratia marcescens*		*E.coli*		*Staphylococcus aureus*		*Pseudomonas aeruginosa*		*Klebsiella pneumoniae*		*Micrococcus luteus*		*Aspergillus flavus*	
	Solvent		Solvent		Solvent		Solvent		Solvent		Solvent		Solvent	
	W	E	W	E	W	E	W	E	W	E	W	E	W	E
- N	--	+	+	+	--	+	+	+	--	+	+	--	--	+
C	+	--	--	--	+	--	++	++	--	+	--	--	--	+
+2N	--	+	--	+	--	+	--	--	+	+	--	--	+	+
+3N	--	++	+	--	--	++	+	--	--	++	--	+	--	--
+4N	+	+	+	--	+	+	+	+	+	+	+	+	+	--

Table 3. Effect of various nitrogen concentrations on the production of antibacterial by *Spirulina plataensis.*

Treatment	*Serratia marcescens*		*E. coli*		*Staphylococcus aureus*		*Pseudomonas aeruginosa*		*Klebsiella pneumoniae*		*Micrococcus luteus*		*Aspergillus flavus*	
	Solvent		Solvent		Solvent		Solvent		Solvent		Solvent		Solvent	
	W	E	W	E	W	E	W	E	W	E	W	E	W	E
- N	--	--	--	--	++	+	+	+	--	+	+	--	--	+
C	--	+	+	++	--	--	++	++	--	+	--	--	--	+
+2N	+	++	--	+	+	++	--	--	+	+	--	--	+	+
+3N	+	++	--	++	--	--	+	--	--	++	--	+	--	--
+4N	+	+	+	+	+	--	+	+	+	+	+	+	+	--

The growth curve of *Spirulina plataensis* was stimulated by all nitrogen supplemented (+2N, +3N and +4N). The maximum growth rate 0.87 µmax.d^{-1} was recorded in culture complemented by +3N and the minimum generation time 43.2G.d^{-1} were shown in cultures deprived nitrogen, at tenth day growth (Table 3, Fig. 2).The dry mass was affected by nitrogen concentrations in parallel with the growth rate. The contents of chlorophyll-a were markedly increased in nitrogen applied, especially at high dose in comparison to control cultures (Table 3).

The data in Table 3 show that, there is a closer relationship between the nitrogen concentrations used in this investigation and the production of antimicrobial activity of *Spirulina plataensis*. Aqueous and ethanol extracts of *S. plataensis* in the high nitrogen supplemented medium (+2N, +3N and +4N) have wide range of antibacterial in comparison to control and nitrogen deprived (–N) cultures. They exhibited significant activity against *S. aureus, E. coli, S. marcescens* and *B. cereus*, *K. pneumoniae* and *M. luteus*, in addition to the fungus *A. flavus*.

4. DISCUSSION

Cyanobacteria produce a wide variety of bioactive compounds, which include lipopeptides, amino acids, fatty acids, macrolides and amides [7]. The results herein revealed that, aqueous and ethanol extracts of the blue green alga *Anabaena circinalis* exhibited antibacterial activity against *Serratia marcescens* and *Escherichia coli*, however it has activity against *Klebsiella pneumoniae* and the fungus *Aspergillus flavus* using only ethanol extracts. Also, the *Nostoc commune* exhibited significant activity against *E. coli, S. marcescens* and *Bacillus cereus* in addition to *K. pneumoniae* and *Micrococcus luteus*. The other blue green alga *Nostoc muscorum* has wide range activity on bacteria Gram-positive bacteria (*Staphylococcus aureus, M. luteus* and *B. cereus*) and Gram-negative bacteria (*Pseudomonas aeruginosa, K. pneumoniae* and *S. marcescens*) in addition to the fungus *A. flavus*. As regards the dominant species of cyanobacteria *Spirulina platensis* under investigation, the aqueous extract of *S. platensis* has antibacterial activity against all species tested except *B. cereus* and *P. aeruginosa*. In this context, cyanobacterial lipopeptides include different compounds like cytotoxic, antitumor, antiviral and antibiotics [7]. Recent researches [36] have also hinted at their possible application to the generation of clean and green energy via converting sunlight directly into electricity. Blue-green algae supplements come in the form of capsules, pills, and powders represent an important part of the food chain in lakes and ponds worldwide [37]. In this work, we trial to nitrogen-enrichment medium and follow the growth, some metabolites and production of bioactive compounds from two dominant cyanobacteria *Nostoc commune* and *Spirulina platensis*. The growth curve of *Nostoc* was enhanced by all nitrogen applied (except –N). The highest enhancement effect was exerted by +3N and +4N. The maximum growth rate (0.221 µ.d-1) as well as minimum generation time (23.9 G.d-1) for *Nostoc* was recorded in culture supplemented with +4N. Chlorophyll a and the dry mass were markedly increased with increasing nitrogen concentration.

The data obtained revealed that, there is no obvious trend between the nitrogen concentrations used in this investigation and the production of antimicrobial activity of *Nostoc commune*. Aqueous and ethanol extracts of *Nostoc* in the nitrogen deprived medium (-N) has wide range of antibacterial in comparison to control and nitrogen supplemented cultures. Kaushik et al. [28] stated that, methanol extract showed more potent activity than other organic and aqueous extracts, no inhibitory effect was found against *Klebsiella pneumoniae* and *Salmonella typhi*. Gram-positive bacteria were found to be more susceptible as compared to Gram-negative bacteria.

Finally, it is concluded from this study that extracts of some cyanobacterial strain showed antimicrobial activity against the pathogens used in the present investigation. Further researches should be made to identify and purify natural product from these cyanobacteria against antibacterial and antifungal activity. Improvement knowledge of the composition, analysis, and the properties of these cyanobacteria with respect to antimicrobial compounds would assist in efforts for the pharmaceutical application.

AUTHORS' CONTRIBUTION

All authors contributed effectively to the data collection, analysis and interpretation of results, drafting and revision of the manuscript.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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TMKARPIŃSKI PUBLISHER

Archives of Biomedical Sciences

ISSN: 2300-7257

ORIGINAL ARTICLE

Seroprevalence of toxoplasmosis among women in Aden city, Yemen

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Received: 21 October 2014; **Revised submission:** 10 December 2014; **Accepted:** 17 December 2014

www.journals.tmkarpinski.com/index.php/abms

ABSTRACT

A total of 670 women attending some private clinics and hospitals in Aden Governorate, Yemen, were examined for toxoplasmosis using Latex, cassette and ELISA tests. The overall seropositive rate of *Toxoplasma* was 64.3%. Seroprevalence of IgG (31%) was higher than of IgM (14%). This contributes that *T. gondii* IgG antibodies in women are reflection of chronic or past infection, while IgM reflect recent and acute *T. gondii* infection. In addition the seropositivity rate IgG + IgM was detected in 22.6%, which reflect the current acute toxoplasmosis. Highest seropositive rate (82.4%) was recorded in age group >38. The low seropositive rate (58.8%) was recorded in age group 27-32. The present study revealed that toxoplasmosis was responsible of 61% of abortion cases in the examined women. The results show that women with more than 3 previous abortions had high (78%) seroprevalence of *T. gondii* antibodies. Higher seropositive toxoplasmosis was recorded among women from sub-urban areas (65.6%) in comparison to women from urban areas (60.8%). Prevalence levels of toxoplasmosis, in relation to education levels, revealed that the rate of infection among illiterate women was high (72.7%). The ownership of animals in relation to the infection was studied; highest seropositive rate (77%) was recorded among women had cats at home. We conclude that toxoplasmosis is one of the public health problems that needs high attention of health authorities in Aden Governorate.

Key words: Toxoplasmosis; ELISA; IgG; IgM; Seroprevalence; Abortion.

1. INTRODUCTION

Toxoplasmosis is one of the most common parasitic zoonoses worldwide [1, 2]. An understanding of the major routes of transmission to humans, and the most sources of infection in a given population is important for the development of effective public health measures for the prevention of toxoplasmosis [3]. Few studies of toxoplasmosis recorded in different areas in Yemen; in Sana'a study of toxoplasmosis in pregnant Yemen women, recorded seropositive 47.4% IgG and 7.7% IgM [4]. High prevalence of 62% and 66% anti-toxoplasmosis IgG antibody by ELISA and Latex assay was already reported in pregnant women attending Thamar General Hospital and private laboratories [5]. Al-Shaebi reported 42.6% seroprevalence of toxoplasmosis by Latex technique and 45.7% by ELISA technique [6]. In Taiz Governorate a study reported 32.5% and 16.0% seroprevalence of toxoplasmosis among disabled children (DC) and apparently healthy children (AHC),

respectively [7]. In Aden in Al-Wahda Teaching Hospital study conducting the histopathological findings in the placenta of fetal death showed that toxoplasmosis is responsible for 3.85% premature rupture of membranes [8]. In addition to that, others [9] recorded that about 2% of chronic toxoplasmosis is responsible of spontaneous preterm birth. Present study aimed to evaluate the prevalence of toxoplasmosis among women in most districts at Aden Governorate, to record the aborting cases due to toxoplasmosis and to evaluate the effect of environmental factors on the transmission of toxoplasmosis.

2. MATERIAL AND METHODS

Study Area

The present study was carried out in Aden Governorate. Eight private laboratories (Al-moraidi, Al-fayroz, Al-yemen, Al-alla, Al-kheer, International Mary Stubs Organization and Al-madeinah Medical Center for Medical Analysis), 3 clinics (Al-fayroz, Al-slahy and Al-shab Charitable) and 2 hospitals (Al-waly and Al-wahda) from 6 district (Crater, Darsaad, Al-shikothman, Al-qahira, Khormakser and Al-mansworah) were visited during the period from July 2011 to May 2012.

Study plan

A total of 670 (661 pregnant and 9 non pregnant) women attending the above health centers were investigated for toxoplasmosis. Blood samples were collected from each woman. Demographical characteristics and information concerning probable risk-factors for toxoplasmosis infection were recorded using a standardized questionnaire. The questionnaire included questions concerning demographical characteristics (age, date of birth, education, marital status, population size of current and childhood residence (city⁄village), eating habits (i.e., eating raw meat or drinking raw milk), and current or past ownership of animals (cats, dogs and rabbits) of the participant.

Methods

In this study three diagnostic tools were used: Toxo-Latex test, Casette test and *Toxoplasma* IgG and IgM ELISA.

Toxo-Latex test

Toxo-Latex test (Almacen, Barcelona, Spain) is a rapid slide agglutination procedure, developed for the direct detection of antibodies-*Toxoplasma gondii* in human serum. The assay is performed by testing a suspension of latex particles coated with antigenic extract of *Toxoplasma gondii* against unknown samples. The presence of anti-*Toxoplasma* antibodies in the samples was tested. Qualitative test was performed according to the manufacturer's instruction.

Cassette test

A nitrocellulose membrane strip containing two test bands (M and G bands) and a control band (C bands) was used. The M band is pre-coated with monoclonal anti-human IgM for detection of IgM anti-*T. gondii* antibody, G band is pre-coated with reagents for detection of IgG anti-*T. gondii* antibody, and the C band is pre-coated with goat anti-rabbit IgG. When an adequate volume of the test sample is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. Anti-*T. gondii* IgM present in the specimen bind to the *T. gondii* conjugates. The immunocomplex is then captured on the membrane by the pre-band, indicating a *T. gondii* IgM positive or reactive test result. Anti-*T. gondii* IgG present in the specimen bind to the *T. gondii* conjugates. The immunocomplex is then captured by the pre-coated reagents on the membrane, forming a burgundy colored G band, indicating a *T. gondii* IgG positive test result. Absence of any bands (M and G) suggests a negative or non-reactive result. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immunocomplex of goat anti rabbit IgG-rabbit. The test performed as described by the manufacturer's instruction.

Toxoplasma IgG and IgM ELISA

The *Toxoplasma* IgG and IgM Kits (Immunospec Corporation, Canoga Park, USA) is based on the ELISA technique. In the assay, calibrators and unknowns are incubated in microtitration wells coated with purified and inactivated *T. gondii* antigen. After incubation and washing, the wells are treated with the conjugate, composed of anti-human IgG anti-bodies and IgM labeled with peroxidase. After a second incubation and washing step, the wells are incubated with the substrate tetramethyl-

benzidine (TMB). An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined. Absorbance was measured at 450 nm. The absorbance measure is directly proportional to the concentration of anti-*T. gondii* IgG and IgM antibodies present. The test was performed as described in the manufacturer's instruction. If the absorbance of the sample is higher than that of the cut-off, the sample is positive for the presence of specific IgG and IgM separately. The ratio between the average OD value of the sample and that of the cut-off was calculated. The sample is considered positive for ratio >1.1 and negative for ratio <0.9.

Statistical analysis

Statistical analysis was performed using SPSS. Data were analyzed using Chi square and Symmetry Measures (Phi).

3. RESULTS

In the present study 670 women sampled were examined for toxoplasmosis out of them 431 were toxoplasmosis positive. The overall seropositive rate was 64.3% (Table 1). Infection with toxoplasmosis in relation to pregnancy period was described in Table 2.

Table 1. Seropositive toxoplasmosis among Yemen women using different diagnostic tests.

Diagnostic test	Total cases examined	Positive (%)	Negative (%)
Latex	330	231 (70%)	99 (30%)
Cassette	132	71 (53.8%)	61 (46.2%)
ELISA	208	129 (62%)	79 (38%)
Total	670	431 (64.3%)	239 (35.7%)

Table 2. Toxoplasmosis in relation to pregnancy period.

Pregnancy period (months)	Total cases examined	Positive (%)	Negative (%)
1-3	476	301 (63.2%)	175 (36.8%)
4-6	163	112 (68.7%)	51 (31.3%)
7-9	22	9 (41%)	13 (59%)

Table 3. Toxoplasmosis among different age groups.

Age groups	Total cases examined	Positive (%)	Negative (%)	Chi	*P*
15 – 20	209	130 (62.2%)	79 (37.8%)	7.394	0.103
21 – 26	260	174 (67%)	86 (33%)		
27 – 32	142	83 (58.5%)	59 (41.5%)		
33 – 38	42	30 (71.4%)	12 (28.6%)		
>38	17	14 (82.4%)	3 (17.6%)		
Total	670	431(64.3%)	239 (35.7%)		

Table 4. Seropositive anti toxoplasmosis IgG and IgM by Cassette and ELISA test.

Test	Sero-positive rate immunoglobulin		
	IgG (%)	IgM (%)	IgG + IgM (%)
Cassette (132)	36 (50.7%)	0	35 (49.3%)
ELISA (208)	69 (53.5%)	18 (14%)	42 (32.5%)
Total	105 (30.9%)	18 (14%)	77 (22.6%)

Table 5. Number of abortion in relation to toxoplasmosis among women.

No. of abortion	No. of women examined	No. of women infected	Positive rate	Chi	*P*
1	125	77	61.6%	23.419	0.001
2-3	115	69	60%		
>3	9	7	77.8%		
Total	249	153	61.4%		

Table 6. Socio-demographic characters of participant women.

Socio-demographic characteristics		Cases examined	Positive (%)	Negative (%)	Chi	*P*
Residence	Urban	408	248 (60.8%)	160 (38.2%)	8.278	0.004
	Suburban	262	152 (68.7%)	103 (39.3%)		
	Total	670	407	263		
Education	Illiterate	194	141 (72.7%)	53 (27.3%)	30.447	0.000
	Primary	134	85 (63.4%)	49 (36.6%)		
	Secondary	275	156 (56.2%)	119 (43.3%)		
	University	67	32 (47.8%)	35 (52.2%)		
	Total	670	414	256		
	Worker	50	23 (46%)	27 (54%)		
	Total	670	435	235		
Ownership of animals	Sheep and goat	99	73 (73.7%)	26 (26.3%)	42.920	0.000
	Cattle and camel	31	19 (61.3%)	12 (38.7%)		
	Birds	26	18 (69.2%)	8 (30.8%)		
	Cats	94	72 (76.6%)	22 (23.4%)		
	Total	250	182	86		
Medium cooked meat		218	141 (64.6%)	77 (35.3%)	31.757	0.000
Raw milk		123	76 (61.8%)	47 (38.2%)		
Total		341	217	124		

Highest seropositive rate (68.7%) was recorded in pregnancy women of 4-6 months pregnancy period and highest seropositive rate (82.4%) was recorded in age group >38. Toxoplasmosis in relation to age groups was also studied (Table 3). Highest seropositive rate (82.4%) was recorded in age group >38 in comparison with other age groups as shown in Table 3.

Data in Table 4 shows the seropositive rate of IgG and IgM antibodies by the two diagnostic test (Cassette and ELISA). The all over seropositive rate of IgG (31%) was highest than seropositive rate of IgM (14%). Cassette test revealed highest seroprevalence of IgG antibodies (50.7%), whereas seropositive of both IgG + IgM antibodies representing 49.3%. The seropositive rate of IgG, IgM and IgG + IgM antibodies detected by ELISA test, was 53%, 14% and 32.5% respectively. Our results show that women with more than three number of previous abortions had the highest seropositive rate of toxoplasmosis (77.8%) in comparison with women had less previous abortions (61.6%) as shown in Table 5.

The analysis of questionnaire described in Table 6, revealed that 408 women were living in urban areas had less seropositive rate (60.8%) in comparison with women from suburban area (68.7%), but it is not statistically significant (P=0.202). The distribution of prevalence levels of toxoplasmosis in relation to education levels was

highly significant (P= 0.0001), the rate of infection among illiterate women was higher (72.7%) than women with high education level (47.8%). In the present study the ownership of animals in relation to the infection was studied, highest seropositive (77%) recorded among women had cats at home, followed by sheep and goats (74%). Similarly, the ownership or contact (with camels and cattle) and pouters also revealed high positive rate: 61.3% and 69.2%, respectively. Results show statistically significant correlation between toxoplasmosis and ownership of cats and other animals (P=0.0001). In addition to that the highest seropositive rate (69% and 62%) was recorded among women eating undercooked meat and drinking raw milk respectively.

4. DISCUSSION

Variations in the incidence of *T. gondii* infection rates from one country to another or even within the same country, has been well documented. Which related to climate, hygiene standards, and eating habits [10-13].

Our results reported a high seropositivity (64.3%) of toxoplasmosis in Aden city. These results are similar to other study in Yemen [5] in which pregnant women attending General Thamar Hospital and private laboratories; it was reported high prevalence of 62% and 66% anti-*Toxoplasma* IgG antibody by ELISA and Latex assay, respectively. However low positive rates of anti-toxoplasmosis IgG and IgM antibodies were reported in some other studies in Yemen [4, 6, 7]. The highest prevalence in our study in comparison with other areas in Yemen might be contributed to the differences in temperature and humidity in Aden which play an important role in the oocyst sporulation. Under environmental conditions such as humidity and warm temperature, oocysts may sporulate and become infective in less than one day [14]. High prevalence rates were also reported in some Arab countries, study in Sudan reported 50.6% seropositive toxoplasmosis among butchers in Khartoum State [15]. Low prevalence rate (31%) was reported for human population in UAE [16]. In addition to that low seroprevalence was recorded from various countries in Asia and Africa [12].

Our findings reported that seropositive toxoplasmosis was increasing with age, but it is statistically not significant (P=0.193). Similarly, studies in Yemen as well as worldwide also reported that toxoplasmosis increase with age [3, 4, 16-18].

In presented studies seroprevalence of IgG was higher than of IgM, similarly in other works [13, 19]. This contribute that *T. gondii* IgG antibodies in women are reflection of chronic infection (past or previous), while IgM reflects recent and acute *T. gondii* infection [4, 5, 20]. Other studies had reported that anti-*Toxoplasma* IgM antibodies commonly persist beyond 6 months, but positive results are very poorly predictive of infections acquired within the previous 2-3 months [21]. At the same time anti-*Toxoplasma* IgG antibodies have been reported to persist for a long time, up to years [22]. Other studies mentioned that IgM antibodies are not commonly detected and IgG antibodies may be undetectable in a minority of cases [23, 24]. These differences in the seropositivity of IgG and IgM also recorded in some countries of Arabian Peninsula; in UAE seropositive IgG and IgM were 29.8% and 5.8% respectively [16], and in Doha, Qatar in the newborn children were 21.9% [13]. In Saudi Arabia 52.1% for IgG and 4.1% for IgM seropositivity were recorded from blood donors in Abha, Asir region, south-western of Saudi Arabia [25]. In Kuwait 53.1% seropositivity for IgG and 13.8% for IgM antibodies were reported [26]. Similar studies worldwide conformed also these results. In Czech, seroprevalence rates of 32.1% for IgG and 2.4% for IgM were reported among blood donors [22]. Furthermore; a high prevalence of IgG in the presence of low prevalence of IgM antibodies might be conceder as an indicator of future prevalence of chronic infection. The detection of both IgG + IgM in the same patients in our study reflect the current acute infection of toxoplasmosis.

Present study revealed that toxoplasmosis was responsible of 61% of abortion among investigated women. Correlation between positivity rate and high numbers of habitual abortion is significant at the P=0.001. Our data demonstrated that high-number of habitual abortion were significantly associated with toxoplasmosis in the studied population, where the prevalence rate increased with a greater number of previous abortions. This

may indicate that *T. gondii* represent the main suspect in pregnancy wastage. These findings were similar to that observed in Iraq, in which 70% of positive pregnant women had history of multiple fetal loss [15]. In other study was reported higher rate (86.7%) in women with previous multiple abortion than we had [5]. In contrast low rate (18.5%) was reported among habitual abortion, compared to 5.9% in the normal pregnancy. In addition to that several studies have reported varying prevalence rates: 9.8% in Hong Kong; 28% in Denmark; 49% in Algeria; and 83% in France [24, 27-29].

Our findings recorded higher seropositivity of toxoplasmosis among women from suburban residence in comparison with women from urban residence, it is statistically significant (P=0.004). This could be attributed to the differences in the life style between residents of suburban and urban areas. In suburban areas of Yemen it is common to animal owners, and stray cats have an easy access to their residents which provide chance to contaminate food and water [3, 17, 20, 30]. In contrast, other studies have shown that prevalence of *T. gondii* is insignificant between rural and urban areas [31]. Our results reported a high seropositive rate (76.6%) of toxoplasmosis among women – cat owners or commonly contact with cats, what agrees with previous studies [32, 33].

Toxoplasmosis among Yemen women is significantly associated (P=0.001) with some hygienic measures such as contacting with other animals rather than cats, eating unwashed raw vegetables, and unwashed fruits, eating medium cooked meat and drinking raw milk. These findings are in agreement with other studies worldwide [34-38]. Some authors assume that about 50% of human toxoplasmosis cases are related to food borne infection [39]. Such analysis also highlights that the risk of *Toxoplasma* acquiring by food varies with cultural and eating habits in different human populations [31].

5. CONCLUSION

Presented studies conclude that seroprevalence rate of *Toxoplasma* is high, and is one of factors responsible for abortion among women in Aden Governorate. It should concern the public health and therefore is need to increase the efforts in diagnosis. It is required to obtain an accurate serological diagnosis of *T. gondii* by combined IgG and IgM anti-*Toxoplasma* serological tests. Management of *T. gondii* and providing of health education to all women in order to prevent primary infection during pregnancy are also needed.

AUTHORS' CONTRIBUTION

Conception and design: NAM; Acquisition of the data, performing the methodology: MAA; Administrative, technical and material supports: NAM and MAA; Analysis and interpretation of data: NAM and MAA; Writing and review the manuscript: NAM. Both authors are involved in drafting the manuscript, read and approved the final manuscript.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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Conference Proceedings of

POLISH SCIENTIFIC CONFERENCE

"ADVANCES IN MICROBIOLOGICAL DIAGNOSTICS"

December 19, 2014, Poznań, Poland

ORGANIZERS:

- Department of Medical Microbiology, Poznań University of Medical Sciences
- Commission of Laboratory-Clinical Medicine of Poznań Society of Friends of Sciences
- Poznań Branch of the Polish Society of Epidemiology and Infectious Diseases
- bioMerieux

CONFERENCE PROGRAM:

10.00	Ceremonial Opening of the Conference Prof. dr hab. Jacek Wysocki, President of Poznań University of Medical Sciences
10.15-10.50	Concert
10.50-11.30	Prof. Dr h.c. Tadeusz Malinski (USA) „Introduction to nanomedicine”
11.30-12.00	Prof. dr hab. Andrzej Denys (Łódź) „Microbiological diagnostics of a bioterrorist attack”
12.00-12.30	Prof. dr hab. Andrzej Gamian (Wrocław) „The use of mass spectrometry to identify the bacterial species”
12.30-13.00	Prof. dr hab. Andrzej Szkaradkiewicz (Poznań) „Rapid microbiological diagnosis”
13.00-13.30	Mgr Ireneusz Popławski (Warszawa) „New technologies and their impact on the work organization in microbiological laboratory”
13.30-14.00	Dr hab. Tomasz M. Karpiński (Poznań) „Advances in molecular diagnostics”
14.00-14.30	Discussion
14.30	Awarding the certificates

Microbiological diagnostics of a bioterrorist attack

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ABSTRACT

Pathogenic microorganisms and their toxins, a kind of weapons of mass destruction, were divided into A, B, C groups depending on morbidity, mortality rate and ability to spread. Group A comprises the smallpox virus, the *Bacillus anthracis*, the plague bacillus, Tularemia toxin, Botulinum toxin, haemorrhagic fever viruses. Group B includes *Brucella* rods, salmonellosis, *Shigella*, *Vibrio cholerae*, Streptococal Enterotoxin B, encelophitis viruses, ricin toxin, rickettsiae.

Effectiveness of biological weapons depends on ease to manufacture the product, no effective vaccine or treatment. Biological weapons may be used in the form of aerosol, contamination of food and drinking water, parcel or letter mailing and suicidal attack. It is very difficult to detect biological weapon attack as there is a latent phase of a disease, from the time of infection until symptom manifestation. Another difficulty is caused by an early symptom syndrome similar for many infectious diseases while patients may already be highly infectious. Suspected disease may be verified only by analysis of epidemiological incidence. Microbiological laboratories are a fundamental component of systems monitoring threat of bioterrorism, diagnosis of microorganisms used is decisive for rescue activities.

Bioterrorist hazard poses a threat to natural environment e.g. spraying anthrax spores, contamination by botulin toxin will cause totally unpredictable consequences in the local population thus monitoring cleanliness of natural environment using new methods of detection is necessary. Interim Biological Agent Detector is a good example of a detector used on ships to monitor air contamination. Biowatch is a program designed to monitor biological contamination spread in air as aerosols; at present the results of sampling analysis may be obtained within 4h. Bioterrorist detection system may be implemented as a network of alarming devices, set off after detecting biological agent. Now molecular biology methods tend to be used more and more often for quick and precise identification of biological pathogens. Polymerase chain reaction (PCR), basic diagnostic method used in genetic diagnostics, enables selective amplification of DNA sections and thus precise diagnosis of bacterial or viral genotype.

Currently, field detection systems for pathogen identification based on new genetic and biosensoric methods are being tested, eg., identification is carried out by a field automated system RAPORT using Real-time PCR method with fluorescence labeling. Mass spectometry is a promising detection method, another method of pathogen detection is flow cytometry. DNA microarray commonly known as DNA chip enables a large number of simultaneous hybridizations on a small dish on which oligonucleotide probes were placed. The investigated DNA is labeled fluorescently under fluorescent microscope. Spots emitting fluorescence signals interpret performed hybridization.

In Poland there is no quick computerized epidemiologic supervising system which would signal suspected epidemiological phenomena. In accordance with the Law on Prevention and Control of Infectious Diseases, in case of suspected dangerous infectious disease a GP, an emergency doctor or a hospital doctor are obliged to report such a case to a district sanitary inspector within 24h. However, the system is little effective. Now, in Poland, only military heath services are prepared for quick identification of biological hazards; Teams for Biological Identification and Center of Diagnostics and Infection Control of Military Institute of Hygiene and Epidemiology in Puławy has been equipped with Laboratory BSL-3.

Application of MALDI-TOF mass spectrometry in bacteria identification

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ABSTRACT

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has recently been introduced in many branches of microbiology as a rapid and accurate identification method comparing traditional phenotypic methods. In this technique unique protein fingerprint of microorganism is obtained and by matching the respective pattern with an extensive database the identity of the microorganism could be determined down to the genus or species level. Moreover, the identification of clinical isolate to a species level is possible after generating in-house database which expand existing databases with adding known species. Clinical laboratories which have a lot of clinical strains can upgrade commercial database by in-house databases to improve identification and speed up results obtained, as MALDI is fast, reliable and of low cost for single sample analysis.

The overview of the principles of MALDI-TOF MS and the examples of different organisms identification by MALDI-TOF MS as well as the sample preparation methods and existing databases will be presented together with future perspectives.

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Rapid microbiological diagnosis

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ABSTRACT

Rapid detection of etiological factor in infection/infectious disease allows for an early implamentation of an effective causal treatment, permitting to save life of a patient. Currently, rapid diagnosis of an infection takes advantage of five basic investigative techniques: Diagnosis employing electron miocroscopy with negative staining (NS-DEM), immunofluorescent techniques (IF), immunoenzymatic techniques (ELISA), technique of rapid optical immunotests (RIPA) and molecular techniques (hybridization techniques and PCR). At the end of 20th century, implementation of negative staining revolutionized the until now used traditional DEM technique, which required a complex and taking few days procedure. Use of heavy metal salt solutions in DEM techniques (NS-DEM) allows for a rapid (15-20 min) visualization of viruses and bacteria in the diagnosed material, originating directly from a patient. Immunofluorescent techniques (IF) enjoy the longest tradition in rapid microbiological diagnosis. Fluorescence of bacterial and viral antigens is detected using the direct technique (DFA) in 15-30 min or the more sensitive indirect technique (IFA) in 30-45 min. Immunoenzymatic tests of ELISA type allow to detect antigens and/or specific antibodies. The result is visualized by a colour immunoenzymatic reaction (time of performance: 1-5 h). The tests assure 100% sensitivity and almost 100% specificity. Immunochromatographic RIPA tests (rapid immunofilter paper assay) allow to visualize pathogen antigen in an optical reaction developing on a test strip in presence of specific antibodies. Duration of test execution amounts to 10-20 min. However, the tests manifest lower sensitivity than that of IF techniques. Molecular diagnosis allowing early detection of genetic material contained in a specific pathogen uses various hybridization techniques and PCR technique, exhibiting extremely high sensitivity (duration of execution: 6 h). Recently, molecular diagnosis of bacterial infections began to use an automated system with application of a DNA microarray, allowing identification of 25 bacterial species in the period of time of 3 h.

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Advances in molecular diagnostics

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ABSTRACT

In modern microbiology an important role play techniques of molecular biology. Particularly important are PCR and hybridization. The polymerase chain reaction (PCR) is used to amplify a single copy or a few copies of a piece of DNA. The result of PCR reaction is generating of thousands to millions of copies of a particular DNA sequence. PCR is used in medical and biological research labs in analysis of genes, DNA cloning for sequencing, DNA-based phylogeny, the diagnosis of hereditary diseases and the detection and diagnosis of infectious diseases. A basic PCR set up requires several components and reagents: DNA template, two primers, Taq polymerase, deoxynucleoside triphosphates (dNTPs), buffer solution, and cations of Mg^{2+} and K^{+}. To visualisation of amplified DNA fragments - PCR products, agarose gel electrophoresis is employed. Currently, are known many variations on the basic PCR technique, e.g. Nested PCR, Multiplex PCR or Reverse Transcription PCR. Classic PCR is used in qualitative research. For quantitative studies is used Real-Time PCR. The procedure follows the general principle of polymerase chain reaction, however the amplified DNA is detected as the reaction progresses in "real time". For the detection of products in quantitative PCR are used non-specific fluorescent dyes that intercalate with any double-stranded DNA (e.g. SYBR Green), and sequence-specific DNA probes consisting of oligonucleotides that are labelled with a fluorescent reporter. Real-Time PCR is applied to diagnostic of infectious diseases, cancers and genetic abnormalities. It can be used also in detection of phytopathogens, detection of genetically modified organisms, genotyping and in quantification of gene expression. PCR methods are especially useful in identification of non-cultivable or slow-growing microorganisms such as mycobacteria, anaerobic bacteria, sexually transmitted pathogens, periodontal pathogens or viruses. Other molecular method used in microbiology is hybridization. Hybridization is the process of combining two complementary single-stranded DNA or RNA molecules and allowing them to form a single double-stranded molecule through base pairing. For the detection of products in hybridization are used colorimetric, chemiluminescent, radioactive or fluorescent methods. Hybridization can be used to detect directly the pathogen's DNA or RNA in patient's tissue and in paraffin slices. One of hybridization type is FISH (fluorescent in situ hybridization) used in cytogenetic, comparative genomic studies, clinical microbiology, and microbial ecology. The continuous development of molecular methods leads to their increasingly broader application in diagnostic medicine, including microbiology.

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Molecular diagnostics of periodontal pathogens

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ABSTRACT

Periodontitis is an infection of the supporting structures of the teeth, with progressive destruction of the periodontal ligament and alveolar bone, leading to tooth loss. Chronic periodontitis belongs to the most frequent inflammatory diseases in humans, which frequency and severity increases with progressing age. In etiopathogenesis of periodontitis the principal role is played by anaerobic bacteria, defined as periopathogens or periodontopathogens. The most important periopathogens include: *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Tannerella forsythia*, *Treponema denticola* and the relative anaerobe of *Aggregatibacter actinomycetemcomitans*. In diagnostics of periodontal pathogens more frequently is used PCR method, which compared to culture is characterized by rapid execution time and greater sensitivity. Samples for research may be gingival crevicular fluid and subgingival plaque. DNA isolation is composed of two processes: lysis and DNA clean-up. Purified DNA can be stored at -80°C. In PCR detection of periodontal pathogens may be used species-specific primers, presented in Table 1. To visualisation of amplified PCR products, agarose gel electrophoresis is employed. In the rapid microbiological diagnosis of periodontal diseases PCR is becoming increasingly important. Simultaneously, the knowledge of the causative agent of periodontitis may affect the taking of appropriate treatment. However, in addition to the PCR should also culture be conducted, without which can not be determined antibiotic sensitivity of periopathogens.

Table 1. Species-specific primers used for detection of periopathogens.

Periopathogen	Species-specific primers	Amplicon size (bp)
Porphyromonas gingivalis	5′- TGT AGA TGA CTG ATG GTG AAA ACC-3′ 5′- ACG TCA TCC CCA CCT TCC TC-3′,	197
Fusobacterium nucleatum	5′-CTA AAT ACG TGC CAG CAG CC-3′ 5′-CGA CCC CCA ACA CCT AGT AA-3′	316
Prevotella intermedia	5′- TTT GTT GGG GAG TAA AGC GGG-3′ 5′- TCA ACA TCT CTG TAT CCT GCG T-3′	575
Tannerella forsythia	5′-GCG TAT GTA ACC TGC CCG CA-3′ 5′-TGC TTC AGT GTC AGT TAT ACC T-3′	641
Treponema denticola	5′-AAG GCG GTA GAG CCG CCG CTC A-3′ 5′-AGC CGC TGT CGA AAA GCC CA-3′	311
Aggregatibacter actinomycetemcomitans	5′-AGA GTT TGA TCC TGG CTC AG-3′ 5′-CAC TTA AAG GTC CGC CTA CGT GCC-3′	593

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Usefulness of alpha-fetoprotein and squamous cell carcinoma antigen as circulating immune complexes (AFP IC and SCCA IC) in the diagnosis of hepatocellular carcinoma in cirrhotic patients

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ABSTRACT

Aim: Current HCC surveillance using total AFP and ultrasound (US) lack the clinical sensitivity for detection of HCC. Total AFP can give false positives, and US is operator dependent and can miss HCC. Than the aim of this study was to estimate usefulness of alpha-fetoprotein and SCCA in immune complexes (AFP IC, SCCA IC) as a novel HCC serum markers in correlation with conventionally used serum AFP for cirrhotic patients of viral and non-viral etiology.

Patients/methods: Serum levels of AFP IC and SCCA IC were measured using enzyme immunoassay: HEPA AFP-IC (XG005) and HEPA-IC (XG003) company Xeptagen. Patients were registered from 2006 in e-HEPAR III database. Serum AFP IC and SCCA IC concentrations were determined in 119 cirrhotic patients with various origin, who were divided into 2 groups. Group 1 (n:90) consisted of cirrhotic patients without HCC, and group 2 (n:29) consisted of cirrhotic patients with HCC confirmed by imaging diagnostics (US and/or CT and/or NMR). For my analysis and calculations it used STATISTICA 9 and Statistical Analysis System (SAS) computer programs.

Results: In both of the groups: HCC and no-HCC, dominate men over 50 years old with cirrhosis of viral etiology (HCV >HBV). In my HCC group (n: 29) only 8 patients (28%) had AFP higher than 400 ng/mL and 11 patients (38%) 100 ng/mL. More than half of HCC patients in our study needs much more useful diagnostic tools. For analysis of relationship between HCC and AFP, AFP IC and SCCA IC the logistic regression was used. The ROC and AUC were calculated. For the estimation of optimal cut-off-point the Youden Index was used. ROC curve analysis shows AUC for AFP: 0.67 (p=0.0015), for AFP IC: 0.61 (p=0.0108), for SCCA IC: 0.620 (p=0.455). The optimal cut-off point for AFP is 13.60, for AFP-IC: 793.54, for SCCA IC – 1096.04. Sensitivity and specificity for each marker were as follows: AFP – 64.29%, 70.89%; AFP-IC – 41.38%, 85.23%. SCCA-IC – 44.83%, 86.52%. According to classification based on optimal cut-off-points complementary use of tests gave the following results: AFP/SCCA IC (AUC 0,73; sensitivity 86%, specificity 64%) i AFP/AFP IC (AUC 0,71; sensitivity 83, specificity 65%)

Conclusions: Single serum AFP testing is not enough accurate for supporting visual techniques in HCC diagnosis. Results of AFP and AFP IC/SCCA IC are not correlated, therefore most accurate for HCC diagnosis is complementary use of both markers together

According to classification based on optimal cut-off-points with I estimated for AFP, AFP IC, SCCA IC seemed to be the significant predictors of HCC. The study results indicated that the best diagnostic ability was obtained using AFP/AFP IC, or AFP/SCCA IC together as a complementary tests.

Monitoring of these biomarkers in high risk groups apart for the repeated US and/or CT/NMR examinations seems to be justified.

Key words: hepatocellular carcinoma (HCC), liver cirrhosis (LC), alfa-fetoprotein (AFP), alfafetoprotein in immune complexes (AFP IC), squamous cell carcinoma antigen in immune complexes (SCCA IC).

The significance of urinary tract infections in diabetes mellitus type 2 post-menopausal patients: epidemiology, risk factors and treatment

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ABSTRACT

Background: Patients with diabetes mellitus (DM) have higher incidence of urinary tract infections (UTIs) compared to those without DM [1]. The infections which accompany DM may have more serious consequences [2], also increasing morbidity in this group of patients [3]. Importantly, women after menopause are also more prone to UTIs due to estrogen deficiency which leads to inhibition of the proliferation of *Lactobacillus* in the vaginal epithelium, pH increase, and vaginal colonization of Enterobacteriaceae [4]. Therefore, identification of the pathogens, cautious consideration of the additional risk factors and the evaluation of the effectiveness of the treatment of UTIs are of vital importance, especially in the group of postmenopausal DM type 2 patients.

Aim: The aim of this study was to assess the occurrence of UTIs in postmenopausal DM type 2 patients, identify the prevailing bacteria and determine their antimicrobial susceptibility checking if the preferred treatment - quinolones – is the most effective one in DM patients. A significant part of the research was establishing which additional factors may influence the onset of the UTI in this particular group of patients.

Material and methods: The study's inclusion criteria were met by 42 female, post-menopausal patients diagnosed with DM type 2, treated at the Department of Hypertension and Metabolic Disorders. Their median age at diagnosis was 64 years (range from 52 to 84) and the median diabetes duration was 6.5 years (range from 0 to 26). The patients were interviewed using specially structured questionnaire which comprised the information on the course of diabetes, method of treatment, accompanying diseases, clinical symptoms of urinary tract infection and the list of UTI risk factors. The severity of comorbidity was assessed using the Charlson Comorbidity Index [5]. The patients were examined, including Goldflam's sign check, and had their medical history analysed. Their uncontaminated midstream urine sample was collected and cultured for identifying pathogens. In the microbiological laboratory Colony Forming Units (CFU) were counted and antimicrobial susceptibility or resistance was tested using European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria [6].

Results: The incidence of UTI among the studied patients was 37.5%. *Escherichia coli* was the most common isolate constituting 50% of the pathogens in the women with bacteriuria. Other Gram-negative bacteria constituted 31.1%. The third most common group of bacteria was Enterococci which occurred in 18,8%. 87,5% of isolated bacteria were susceptible to all tested quinolones. 100% susceptibility was reported for Ciprofloxacin, Levofloxacin, Moxifloxacin and Pefloxacin. As far as the additional risk factors are concerned the significant ones which seemed to promote UTIs were: urinary incontinence, microalbuminuria and hyperlipidemia. Microangiopathic complications such as retinopathy and nephropathy 4.9 times increased the risk of UTI. The patients with UTI had meaningfully lower Glomerular Filtration Rate (GFR) and higher comorbidity assessed with Charlson Comorbidity Index compared to these without bacteriuria. However, such variables as age, duration of diabetes, fasting blood glucose, and the quality of diabetic control measured with HBA1c seem to have no significant impact on the occurrence of UTI.

Conclusion: As UTIs may impair renal function and also lead to systemic complications, especially in diabetic post-menopausal women, they should be treated with special attentiveness. These patients, and particularly the ones presenting microanghiopathic complications, urinary incontinence, microalbuminuria

and lower GFR should be educated and closely monitored as such factors make them even more prone to UTIs. As far as the treatment is concerned quinolones should still be used as medication of choice in urinary tract infections in this group of patients.

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