Extended-spectrum beta-lactamase *Klebsiella pneumoniae* and carbapenemase-producing *Enterobacter spp*. in algae products marketed as health suppplements

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Introduction

Chlorella, Spirulina and Super Greens are three popular neutraceutical food supplements available widely in Ireland

Nutritional supplements are popular worldwide claiming many health benefits, however, these claims are unsubstantiated by clinical studies.[1] These ready to eat foods are most commonly consumed mixed in water or smoothies or sprinkled on foods and consumed without cooking or other heat treatment.

- Chlorella is natural green algae
- Spirulina is filamentous microalgae
- Supergreens contain Chlorella and Spirulina as well as wheat grass and barley grass

Figure 1. Illustration of algae supplements

Production is possible by a number of methods: open systems, raceway ponds and closed systems, the latter preventing contamination from the environment.

Most large-scale commercial production is by open systems.
Spirulina and Chlorella are sold as dietary supplements, without any kind of processing except drying. Most producers of micro-algal products are located in Asia and Australia with China being one of the leading producers of Spirulina and Chlorella.[2-3]

Figure 2 1. Example of an open production system

In April 2015, the Food Safety Authority of Ireland (FSAI) announced recall of a batch of a specific brand of Chlorella powder due to contamination with *Salmonella* Rissen.

Study Aims:

1. Test recalled and related products for other *Enterobacteriaceae spp.*
2. Test isolates for multi-drug resistnance

Methods

8 samples were selected for testing:

- five Chlorella
- two Spirulina
- one Super Greens

Twenty-five gram samples of the supplements were diluted in 225g of peptone buffer solution and cultured for 24 hours at 37°C
They were then inoculated onto

- Columbia Blood Agar
- Mueller-Hinton Agar
- selective chromogenic agars for vancomycin-resistant *Enterococci*, extended-spectrum beta-lactamases, carbepenemase-producing enterobacteriaceae, and methicillin-resistant *Staphylococcus aureus*

Incubated at 37°C for a further 24 hours
Colonies were identified using MALDI-TOF MS (Brucker)
Susceptibilities performed using EUCAST disk diffusion methods.
Zone diameters were interpreted according to EUCAST criteria.

Isolates that were ESBL-producers or had increased meropenem MICs were evaluated by molecular methods.
PCR using specific primers was used for detection of

- CPE (KPC, OXA-48, VIM, IMP, NDM, GES, IMI, OXA-23, OXA-51, OXA-24/40, OXA-58)
- CTX-M (Group 1, 2, 9, 8, 25)
- Plasmid encoded ampC (ACC, ACT, CIT, DHA, FOX)

Results

Cultured from all samples

- *Enterococcus faecium*
- Range of *Enterobacteriaceae*
- Anaerobes

Cronobacter sakazakii (4 samples)
Bacillus cereus (2 samples)

Sample	Isolates
A Chlorella	*Enterobacter cloacae*
	Enterococcus faecium
	Cronobacter sakazakii
	Citrobacter sedlakii
	Clostridium butyricum
	Clostridium perfringens
B Chlorella	*Cronobacter sakazakii*
	Escherichia coli
	Enterococcus faecium
	Enterobacter cloacae
	Clostridium perfringens
C Spirulina	*Enterococcus faecium*
	Enterobacter cloacae
	Clostridium cochlearium
	Clostridium perfringens
	Clostridium tertium
	Lysinibacillus sphaericus
	Bacillus cereus
D Spirulina	*E.faecium*
	Cronobacter sakazakii
	Enterobacter cloacae
	Clostridium cochlearium
	Clostridium perfringens
	Bacillus cereus
	Bacillus badius

Sample	Isolates
E Super Greens	*Enterobacter kobei*
	Acinetobacter baumanii
	Klebsiella pneumoniae
	Cronobacter sakazakii
	Enterobacter cloacae
	Enterococcus faecium
	Clostridium tetani
F Chlorella	*Klebsiella pneumoniae*
	Enterobacter asburiae
	Enterococcus faecium
	Enterobacter cloacae
	Klebsiella pneumoniae
	Clostridium tetani
	Clostridium glycolicum
G Chlorella	*Enterobacter cloacae*
	E.faecium
	Enterobacter cloacae
	Klebsiella pneumonia
	Anaerobes (unidentified)
H Chlorella	*E.faecium*
	Enterobacter cloacae
	Enterobacter cloacae
	Clostridium butyricum
	Clostridium cochlearium

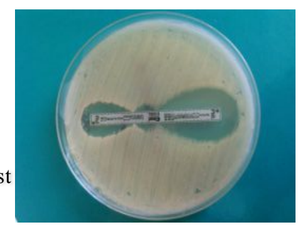
Table 1. Organisms isolated by sample

***Klebsiellla pneumoniae* – Sample E**

Resistant
amoxicillin, amoxicillin-clavulanate, gentamicin, cefotaxime, and cefpodoxime.
Intermediate
ciprofloxacin
Susceptible
amikacin, piperacillin-tazobactam, meropenem, cefoxitin and ertapenem.
Meropenem MIC was 0.023mg/L

Molecular characterisation – **CTX-M Group 9**

Figure 3. *Klebsiella pneumoniae* PM:PML Etest showing phantom zone.



***Enterobacter kobei* – Sample E**

Resistant
amoxicillin, amoxicillin-clavulanate, piperacillin-tazobactam, cefotaxime, ciprofloxacin, ertapenem, cefoxitin and cefpodoxime.
Susceptible
gentamicin, amikacin and meropenem.
Meropenem MIC 1.5mg/L
Molecular characterisation- **OXA-51**

***Klebsiella pneumoniae* –Sample F**

Resistant
amoxicillin, cefotaxime and cefpodoxime.
Susceptible
amoxicillin-clavulanate, piperacillin-tazobactam, gentamicin, amikacin, ciprofloxacin, meropenem, ertapenem and cefoxitin
Phenotypic ESBL (CPD:CPDCV = 26:12)
Molecular characterization- blaCTX-M not detected (presumed blaSHV or blaTEM)

Conclusions

Clinicians caring for vulnerable patients should be aware of the risk with regard to the use of these supplements.

More extensive surveys are required to establish frequency of contamination and patterns of use of these supplements to determine need for a code of practice or regulation in relation to the production and sale.

References

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