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A laboratory-based evaluation of tube blocking and microbial risks associated with one blended enteral feed recipe

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Angela Madden conceived the idea, designed and supervised the overall study, contributed to data collection and led the writing of the manuscript.

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Elise Chen and Lee Jerome made the feeds, evaluated feed waste, undertook the tube blockage evaluation and contributed to the manuscript.

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Each of the authors declares that they have no conflicts of interest.

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Abstract

Background: Concerns associated with blended enteral feeds include risk of blocked tubes and microbial contamination but evidence is limited. This lab-based investigation aimed to examine these risks in a blended feed providing a nutritionally adequate intake for a hypothetical patient.

Methodology: One blended feed recipe was made using three different methods (professional, jug and stick blenders) and three storage procedures. Feed samples were syringed via 10, 12 and 14 French enteral feeding tubes and blockages and time taken recorded. Feed samples were diluted, plated on agars, incubated and bacterial colony forming units (CFU) counted. After storage at -80^{o}C, identification was undertaken using 16S rRNA PCR sequencing.

Results: Two blockages occurred during 27 administrations of feed made using a professional blender but were resolved with water flush. No blockages occurred with the 14 French tube and administration was quicker with wider tubes ($P<0.00001$). There was no significant difference between total bacterial CFU of feeds prepared using different methods ($P=0.771$) or stored differently. The genus of bacteria identified included *Enterococcus, Bacillus,* lactose-fermenting *Enterobacteriaceae, Pseudomonas and Staphylococcus*. Pathogens, such as *Clostridium* spp., *Salmonella* spp. and *Vibrio* spp., were not identified by phenotypic tests used. Sequencing identified *E. coli, Shigella species, Streptococcus lutetiensis* and *Staphylococcus epidermidis*.

Principal conclusions: This evaluation found no risk of tube blockages when one blended feed recipe made using three methods was delivered via a 14 French tube. There is concern about bacterial contamination although this was not influenced by the methods of preparation or storage used in this study.

Introduction

Usual practice in enteral tube feeding is to provide nutrition through commercially prepared, nutritionally-complete liquid feeds [1]. However, there is increasing patient and carer-led interest in providing nutrition using blended or liquidised food that is prepared and administered at home [2,3]. Reported benefits associated with blended diets include improvements in reflux and bowel problems and empowering patients and carers without 'medicalizing' feeding [4-6]. A number of concerns about health risks associated with blended diets have been described [1,7-10] and these include nutritional inadequacy, blocked feeding tubes and food-borne infection [1,11].

However, there is little systematically reported evidence to support or refute these concerns [5,8,9,12]. Peer-reviewed guidance on how blended diets should be prepared or administered is available but acknowledges the limited evidence [13-15]. Advice from patient and carer-led websites is also available [2,3]. However, there is little evidence that guidance or advice has been evaluated in terms of risk of tube blocking or microbiological load.

The primary aims of this series of laboratory-based studies were to examine the risk of blockage of feeding tubes and the microbiological load associated with a blended feeding regime providing a nutritionally adequate intake for a hypothetical patient. Secondary aims included evaluating practicalities such as time to deliver the feed and food waste associated with blending food. As different terminology, including liquidised, blenderised and pureed, is used to describe these feeds, the term 'blended feeds' is used in this paper as this is commonly used by patients and carers.

Methods

Blended feed recipe

A recipe for a blended diet was developed based on ideas for ingredients shared by patients and carers [2,3] and designed to meet the estimated nutritional requirements [16-23] for a hypothetical man aged 70 years weighing 68 kg, with body mass index of 22.5 kg/m^2 and low physical activity (Table 1). This person was arbitrarily chosen because home enteral feeding is more prevalent in those aged ≥60 years and ≤5 years [24] and producing a larger volume of feed for a hypothetical adult was more practical for the procedures described below. It was assumed that apart from requiring tube feeding, the man was otherwise in good health and had no other clinical conditions that might impact on his intake i.e. able to tolerate lactose, cow's milk protein etc. Where possible, ingredients that were considered lower risk from a food hygiene perspective were used, i.e. not raw meat, fish or eggs. Providing estimated energy and macronutrient requirements in the feed recipe was prioritised because micronutrients could be more easily supplemented if needed. Nutritional composition was determined using web-based nutrient analysis software (Nutritics.com, Dublin).

Blending procedures

The blended feed was made by combining all the ingredients using one of three different methods using (A) a professional blender (Vitamix Professional Series 750) and extra fine sieve and cleaned using solution from sterilising tablets (Milton Pharmaceutical UK Limited); (B) jug blender (Kenwood Series BL430) and standard sieve and cleaned with cold water; and (C) stick blender (Kenwood Series HB6600) without sieving and cleaned with hot water and supermarket regular washing up liquid. The feeds were made by students studying nutrition and dietetics, who had passed a level two food safety certificate, and production was undertaken in a diet laboratory adhering to strict hygiene procedures and under staff supervision. After making, the feeds were divided into three samples which were treated as follows to mimic possible home storage scenarios: (X) no storage, transferred immediately to the microbiology lab for analysis; (Y) stored in a domestic fridge at approximately 4^o C for 24 hours followed by 2 hours at ambient temperature; (Z) stored in a domestic fridge at approximately 4^o C for 48 hours followed by 4 hours at ambient temperature. These procedures were designed to reflect optimum practice with minimal opportunity for microbial growth (X), an approach suggested as good practice [15] (Y) and a high-risk procedure (Z) which deviated from this [15]. The residue remaining on all utensils for feeds A, B and C and the unsieved fraction (i.e. waste) from feeds A and B were weighed to determine total waste.

The nutritional composition of the remaining feed (i.e. total recipe – [residue remaining on utensils + unsieved fraction]) was then compared with the estimated nutritional requirements making an assumption that the proportions lost would be comparable for all nutrients.

Tube blockages and feeding time

Immediately after making, 60 ml samples from each of the three feeds, ABC, were administered in triplicate using a 60 ml enteral compatible syringe through three different sized clean enteral feeding tubes (10, 12 and 14 French; Corpak MedSystems, Illinois, USA) into an empty container. 20 ml water was administered after every 60 ml of feed and a 10 second break was given after every 20 ml of feed to mimic the effect of chewing and swallowing in normal eating. The number of blockages, attempts to unblock, time taken to administer the feed and the researcher's observations of the process were recorded. The administration was repeated with a standard 1 kcal/ml formula feed (Nutrison, Nutricia). In order to consider the physical strength required to administer the bolus feeds, the left and right handgrip strength of two researchers were measured using a digital grip-strength dynamometer (TKK Takei 5501 Grip-D, Tokyo, Japan) following the method of España-Romero et al. [25] and compared against normative values [26].

Microbial load

Samples from each of the three feeds, ABC, at each of the three storage timepoints, XYZ, were diluted, spread on seven types of agar and incubated aerobically (except Columbia blood agar: anaerobically) at 37°C (except mannitol yolk polymyxin at 30^{o}C) (Table 2). Total colony forming units (CFU) were counted in triplicate with 10% blind checked for accuracy by a second researcher. Using CFU/g determined during presumptive testing, the microbial load in each feed of (i) *Bacillus cereus* was compared with guidelines for interpreting results for enumeration of bacterial pathogens and (ii) *Enterobacteriaceae* was compared to guidance on the interpretation of results for hygiene indicator organisms in ready-to-eat foods [27].

Microbial identification

Bacterial colonies of unique morphologies were randomly selected for identification by Gram staining, oxidase test, catalase test and API 20NE strips (bioMerieux), as well as re-streaked onto nutrient agar plates (Oxoid, UK) for pure culture and stored at -80°C. Resuscitated pure cultures on nutrient agar were subcultured in 10 mL nutrient broth (Oxoid, UK) and

incubated at 37°C for 24-48h for genomic DNA extraction using the GenElute Bacterial DNA Kit (Sigma Aldrich, UK). Each PCR was carried out with 10-100ng of DNA template, 0.5 μM each of 16S rRNA universal primers 27F (5' AGAGTTTGATCMTGGCTCAG 3') and 1492R (5' TACGGYTACCTTGTTACGACTT 3'), 200 μM of each dNTP, 0.02U of Phusion DNA polymerase, and 1 x Phusion Green HF buffer (New England Biolabs, UK). Amplicons with an expected amplicon size of 1.4 kb, sized by gel electrophoresis, were column purified with Monarch PCR & DNA Cleanup Kit (New England Biolabs, UK) and sequenced using the same 16S rRNA universal primers. Sequence analysis was carried out with CLC Workbench (Qiagen) and BLASTn [28].

Statistical analysis

The effect of the preparation method and tube size on blockages was analysed descriptively. After testing for normality, multivariate two-way ANOVA was used to examine differences in time taken to deliver (i) the blended feeds delivered via tubes of different diameter, (ii) the blended feeds prepared using the three different methods and (iii) the three blended feeds and standard formula feed. ANOVA was also used to compare CFU across groups for differences associated with preparation method and storage time and P values <0.05 were considered to be statistically significant.

Ethical permission was not required.

Results

Nutritional analysis and waste

The feed recipe provided >95% of estimated requirements for energy and all nutrients except for selenium and vitamin D (Table 1). The total waste for feed A (extra fine sieve) was 942 g (32%), for feed B (standard sieve) was 891 g (31%) and for feed C (unsieved) 29 g (1%). After deducting 32% for the total waste, the remaining feed provided <95% of estimated requirements for energy, fibre, iron, zinc, selenium, vitamins A, D, E and B_6 and fluid (Table 1).

Tube blockages and feeding time

Two tube blockages occurred during 27 feed administrations and both were associated with feed A, i.e. prepared using the professional blender and extra fine sieve. The blockages occurred once each with 10 and 12 French tubes but a single 10 ml water flush was sufficient to resolve both blockages. No blockages occurred with feeds B or C or when using a 14 French tube. The time taken to deliver one 60 ml bolus varied between 46-137 seconds excluding the 20 second rests. There was no significant difference between the time taken to deliver feeds prepared using different methods (P=0.987) but the time decreased significantly as tube size increased (P<0.00001) (Table 3). No blockages occurred with the standard formula feed for any tube diameter and it was significantly quicker to deliver the standard feed via the three tubes than the blended feeds (P=0.00001) (Table 3). The researchers observed that substantial force was required to deliver the bolus feeds using the syringe especially with the smaller tubes. Their mean handgrip strength was 17.7 and 18.3 kg (left) and 18.9 and 18.1 kg (right) respectively; all values were <10% for age and gender normative values [(26)].

Microbial load and identification

There was no significant difference between total bacterial CFU of blended feeds prepared using different methods with values varying widely (A=46.6±48.3; B=53.5±49.3; C=36.3±31.8; P=0.771). The impact of storage time on bacterial CFU varied with increase in colonies on some agars but, overall, was not significantly different (feed A, P=0.091; B, P=0.764; C, P=0.263; Table 2). The genus of bacteria identified included *Enterococcus, Bacillus,* lactose-fermenting *Enterobacteriaceae, Pseudomonas and Staphylococcus* and were similar for all three methods of feed preparation (Table 4). Pathogens, such as *Clostridium* spp., *Salmonella* spp. and *Vibrio* spp., were not identified by the phenotypic tests used.

Potentially clinically significant Gram negative, non-Enterobacteriaceae taxa identified using API 20NE strips included *Pseudomonas alicaligenes* from feed prepared using method A and *Pseudomonas luteolin*, *Pseudomonas fluorescens* and *Pseudomonas putida* from feed prepared using method C. Of 16 cryogenically preserved cultures, only 10 were viable and genomic DNA could be extracted for PCR. Sequencing of 16S rRNA gene identified *E. coli*, *Shigella species*, *Streptococcus lutetiensis*, *Staphylococcus epidermidis*, *Staphylococcus warneri*, and *Lactobacillus paracasei* subsp. tolerans. The presumptive bacterial load of *Bacillus cereus* of all blended feed samples was within the borderline category defined by the Health Protection Agency [(27)], i.e. CFU/g between 10^3 and $\leq 10^5$, regardless of preparation or storage procedure. However, bacterial load of *Enterobacteriaceae* of approximately half the blended feeds was categorised as unsatisfactory, i.e. CFU/g $>10^4$ with no clear pattern of association with preparation or storage method.

Discussion

This laboratory-based evaluation is the first known systematic examination of the combined risks of tube blocking and microbial load of a blended feed. Only one recipe, based on low microbial risk, was examined and different results would be anticipated if different ingredients were used. However, the results provide useful information that is relevant to service users and healthcare professionals working in this field.

The total recipe met the estimated nutrient requirements of the hypothetical man it was designed for except for selenium and vitamin D and this deficit could easily be made up with supplementation. However, the process of sieving resulted in almost one third of the total recipe weight being lost in spite of determined efforts, including repeat blending, to sieve the feeds. Interestingly, the weight of feed lost using different blenders and sieves was comparable. The estimation of nutritional adequacy of the feed remaining after sieving (Table 1) makes an assumption that the losses of all nutrients are equivalent to the sieved weight loss which is probably incorrect. Reports on the effects of sieving on individual dry ingredients, e.g. grains and legumes, indicate this is associated with loss of micronutrients [29] but comparable data are not available for blended feeds. Selecting ingredients that would provide less fibre may reduce sieved losses and requires exploration. Although sieving has been recommended for blended feeds [10,13], this practice was not reported by patients and carers participating in a qualitative study [6].

The results from the present study indicate that the risk of blocking a 14 French enteral feeding tube when administering this blended feed recipe prepared using all three methods, including the stick blender without sieving, is low. This is compatible with anecdotal comments posted on social media by carers who routinely use blended feeds without major problems associated with blockages [30]. The findings may not be transferrable to a clinical situation and are limited to the recipe investigated as other foods may increase the risk of tube blocking. However, the ability for the feed made using a stick blender to be successfully delivered by syringe bolus without blocking the tubes is important because a high-powered professional blender is frequently described as necessary or desirable [2,3,30] but this equipment is considerably more expensive than a stick blender, i.e. ±£400/459€/510$ compared to ±£30/33€/38$ (2019 prices). The consistency of feed produced using the three methods varied with the thickest being produced using the professional blender and the colour also varied with production method suggesting that the blending processes resulted in

different levels of plant cell breakdown; this may have implications for intestinal absorption of micronutrients. Although the time associated with administering the feed via the 14 French tube was significantly quicker than for narrower tubes, extrapolating to delivering the whole day's feed in addition to making it, would be considerably more time consuming for carers than using a standard formula. This additional time commitment has been reported by carers but accepted as necessary to enable blended feeding [6,31]. In addition, the force required to empty the syringe may be challenging for frail carers. Both these issues require further investigation so that carers can be best supported.

The bacteria genera identified were expected due to the use of non-sterile food even though low risk ingredients were used and the feeds were made in an area with strict hygiene procedures and by researchers with a certificate in food hygiene. Whilst the enumeration of *Bacillus cereus* was categorised as borderline using Health Protection Agency [27] criteria, that of *Enterobacteriaceae* was unsatisfactory in approximately half of the blended feeds with no clear picture emerging of safer or less risky preparation or storage procedures. This raises concern about the microbial safety of the feeds overall because *Enterobacteriaceae* is considered an indicator organism, i.e. suggesting an overall poor hygiene status. Comparable microbial loads of blended feeds have been previously reported [10,32-34] and is inevitable in blended feeds made from non-sterile ingredients and in non-sterile conditions. Carvalho *et al.* [35] identified blenders, work surfaces, jugs and sieves as 'dirty zones' with potential for microbial contamination in an evaluation of enteral feeding in hospital. These are also likely to be a source of contamination in domestic kitchens which, in addition, may also be the site of non-food practices [36,37] which have the potential to further increase risk. While the identification of bacterial species from a limited selection of culturable colonies was helpful for gauging the presence of pathogens, a more comprehensive screen will be needed to accurately determine pathogen load and transmission in blended feeds. Culture of bacterial species with fastidious growth requirements (e.g. strict anaerobes) should also be considered. Further genetic analyses will be needed if one was to consider antibiotic resistant bacteria.

While sterile formula feeds are associated with least microbial risk, sterile production of blended feeds would be hard to achieve in a domestic setting. In addition, complex procedures need to be reconciled with the concept of home blended feeding being 'just food' [31] and the social benefits of being included in a family meal [6] which are considered highly important by those choosing to use this method of feeding. Feed sterility may not be an

appropriate goal for those who required enteral tube feeding but are otherwise physically stable and not immunocompromised. It should be noted that 433 parents of blended-fed children reported their children had fewer gastrointestinal symptoms with blended feeds than with standard formula and, when these occurred, attributed them to the child's medical condition rather than food-borne illness associated with feeding [38]. Similarly, improvements in bowel habits associated with receiving blended feeds have been reported [6,39]. The positive role of microbes in gastrointestinal health [40] needs consideration as these may be contributing to some of the improvements reported by those using or preparing blended feeds. This needs to be balanced against potentially life-threatening risks associated with foodborne illness [41]. While randomised controlled trials of blended diets may be ethically challenging due to these risks, future studies based on systematic clinical observations and risk-benefit modelling are needed.

This study is limited to one hypothetical blended feed recipe, its laboratory-based design and by the absence of testing for *Listeria* spp. The study only tested a bolus method of administering the feed and did not investigate the use of continuous feeding using a pump. A more extensive study design that allowed preparation method, equipment cleaning regime and storage to be independently tested and including a wider range of microbial evaluations, e.g. identifying at genus level using a MALDI-TOF mass spectrometer, might provide more useful information that could inform guidelines for those patients and carers who decide to proceed with blended feeding.

In conclusion, this small laboratory-based evaluation of one blended feed recipe found little risk of tube blockages associated with delivery via a 14 French tube and this was not influenced by the method of feed preparation. The findings raise potential concern about the microbial load of blended feeds but this was not influenced by the method of preparation or storage used in this study. The time taken to deliver blended feeds via enteral tubes was significantly longer than for a standard 1 kcal/ml formula feed and this needs to be considered by carers. Sieving feeds was associated with considerable food waste and, for the recipe evaluated, was unnecessary as risk of tube blockage was not increased with unsieved feeds.

Transparency declaration

The lead author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.

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Table 1

Recipe and calculated nutritional composition of blended diet and comparison with estimated requirements for hypothetical man

Ingredients and weight			
Whole fat milk	855 g	Avocado	133 g
Cooked brown wholegrain rice	570 g	Water	95 g
Raw tomatoes	532 g	Feta cheese, regular not low fat	55 g
Lettuce	342 g	Red wine vinegar	8 g
Chick peas, canned, drained	312 g		

Nutrient	Quantity in feed	Estimated requirement (ER)[a]	Adequacy of total recipe (% ER)	Adequacy after deducting 32% waste (% ER)[b]
Energy (kcal)	2142	2151	100	68
Protein (g)	90	53	>100	>100
Carbohydrate (g)	259 [48% energy]	50% energy	96	96
Fat (g)	83 [35% energy]	35% energy	100	100
Fibre (g)	38	30	>100	86
Sodium (mg)	982	<2359	Within target	Within target
Calcium (mg)	1595	700	>100	>100
Magnesium (mg)	560	300	>100	>100
Iron (mg)	10	8.7	>100	78
Zinc (mg)	13	9.5	>100	93
Selenium (μg)	37	75	49	34
Iodine (μg)	282	140	>100	>100
Vitamin A (μg)	805[c]	700	>100	78
Vitamin D (μg)	0.3	10	3	2
Vitamin E (mg)	16	13[d]	>100	84
Vitamin K_1 (μg)	478	70[e]	>100	>100
Thiamin (mg)	1.9	0.9	>100	>100
Riboflavin (mg)	2.6	1.3	>100	>100
Niacin[f] (mg)	39	16	>100	>100
Vitamin B_6 (mg)	1.8	1.4	>100	87
Folic acid (μg)	497	200	>100	>100
Vitamin B_{12} (μg)	8.3	1.5	>100	>100
Vitamin C (mg)	146	40	>100	>100
Water (g)	2404	2500	96	65

[a] Estimated requirements based on: energy, estimated average requirement using physical activity level of 1.49 [16]; macronutrients and most micronutrients, reference nutrient intake [17]; fibre [18]; sodium based on <6 g salt [19]; vitamin D [20]; vitamin E [21]; vitamin K [22]; fluid [23].

[b] Estimated by deducting 32% from quantity in feed to account for sieved losses from feeds A and B.

[c] Retinol equivalents.

[d] α-tocopherol.

[e] Phylloquinone only.

[f] Nicotinic acid equivalents.

Table 2

Impact of storage time on log colony forming units per g of blended feeds prepared using different methods and grown on seven agar types after incubation

Agar and presumptive bacteria selected	Incubation	Method	Storage		
			X	Y	Z
Baird Parker: *Staphylococcus aureus*	Aerobic at 37°C	A	3.52	4.07	4.30
		B	3.45	2.22	2.52
		C	2.70	2.22	2.22
Cetrimide: *Pseudomonas aeruginosa*	Aerobic at 37°C	A	4.39	4.81	4.51
		B	2.52	4.12	3.89
		C	3.71	4.74	5.30
Columbia blood: Non-selective	Anaerobic at 37°C	A	4.25	4.46	5.41
		B	4.43	5.19	5.39
		C	5.67	5.50	5.38
Kanamycin: *Enterococci species*	Aerobic at 37°C	A	3.54	4.53	4.64
		B	3.58	3.50	3.22
		C	3.43	3.56	4.39
MacConkey A: *Non-lactose fermenting Enterobacteriaceae*	Aerobic at 37°C	A	3.92	4.46	5.24
		B	4.11	4.36	3.12
		C	ND	4.10	5.43
MacConkey B: *Lactose fermenting Enterobacteriaceae*	Aerobic at 37°C	A	3.22	3.26	3.58
		B	4.41	3.12	4.19
		C	ND	3.79	3.86
Mannitol yolk polymyxin: *Bacillus cereus*	Aerobic at 30°C	A	4.61	4.49	4.50
		B	3.18	4.02	4.63
		C	4.61	4.52	4.79
Nutrient: Non-fastidious organisms	Aerobic at 37°C	A	4.60	5.68	5.70
		B	3.68	4.37	4.91
		C	5.73	5.27	4.50
Mean ± SD		A	4.01 ± 0.53	4.47 ± 0.68	4.74 ± 0.69
		B	3.67 ± 0.65	3.87 ± 0.91	3.99 ± 0.98
		C	3.23 ± 2.25	4.21 ± 1.05	4.48 ±1.07

Storage: X = plated immediately; Y = 24 hours in fridge + 2 hours at ambient temperature; Z = 48 hours in fridge and 4 hours at ambient temperature
Method: A = professional blender; B = jug blender; C = stick blender
ND: not detected (zero used for statistical analyses)
ANOVA across three storage times: method A, P=0.091; method B, P=0.764; method C, P=0.263.

Table 3

Time taken to deliver one 60 ml bolus of feed prepared using three different blending methods and a standard formula feed through tubes of different diameter, mean ± SD

Tube size in French[a] gauge (external diameter)	Method of feed preparation[b]			Standard formula feed[c] (1 kcal/ml)
	A: Professional blender + extra fine sieve	B: Jug blender + standard sieve	C: Stick blender + no sieve	
10 (3.3 mm)	95.7 ± 10.5	107.7 ± 8.5	105.3 ± 10.7	40.3 ± 0.6
12 (4.0 mm)	105.7 ± 14.4	114.0 ± 21.0	107.3 ± 10.7	35.3 ± 2.5
14 (4.6 mm)	65.0 ± 4.8	50.2 ± 10.2	57.9 ± 3.3	27.3 ± 2.1

Values exclude rest time during bolus delivery

[a]ANOVA across three tube sizes for three blended feeds, P<0.00001

[b]ANOVA across three methods of blended feed preparation, P=0.987

[c]ANOVA across three blended feeds and standard formula feed, P=0.00001

Table 4

Genus of bacteria identified in blended feeds prepared using three methods

Genus	Method of feed preparation and cleaning		
	A: Professional blender + extra fine sieve + solution from sterilising tablets	B: Jug blender + standard sieve + cold water wash	C: Stick blender + no sieve + hot soapy water
Enterococcus	3	3	2
LA fermenting Enterobacteriaceae	1	1	3
Staphylococcus	2	3	3
Pseudomonas	1	3	1
Bacillus	2	2	0