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Original Research Article

Anti-microbial susceptibility pattern of infant feeding bottles from bottle fed infants

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ABSTRACT

Background: Breastfeeding, which has been declining in the recent years because of advances in science. Thought the importance of breastfeeding have been studied, the implementation of the same is being difficult, because of lack of awareness among mothers, health care workers etc.
Methods: Consent was taken from the parents who fed their babies with infant feeding bottle and explained about the necessity to obtain culture of the bottles and nipples. Swab was inoculated on the MacConkey agar culture. Antibiotic susceptibility testing will be done for all the isolates.
Results: This study showed that 32% were initiated on bottle feeding before 6mo of age and 45% during 6-12mo of age and 23% after age of 1 yr. The most common presenting illness were diarrhea (43%) followed by respiratory illness (39%). Bottle feeding has contribution to the malnutrition and showed that adequate cleaning practices were followed by only 41 % of mothers and 9% of mothers had poor cleaning practices. The incidence of illness is 60. 7% in only top fed babies compared to babies who are both breast fed and bottle fed 39.3%. The most common isolate found was E-coli 45% (24) followed by. Kliebsella 24% (12), Staphylococcus 16% (9), CONS 9% (5), Enterobactereccia 3% (2) and Pseudomonas 0.8% (1). 52% of the sample showed fungal isolation positive.
Conclusions: The incidence of bottle feeding was found more common in working women(53%) compared to housewife(19%). Illiteracy of the family contributed to the over dilution of top milk .most common organism is *E-coli*.

Keywords: Culture, Exclusive breast milk, Feeding bottle, Infant feeding practice, Malnutrition, Microbiology

INTRODUCTION

The benefits of breastfeeding have been well recognised. Education and promotion of breastfeeding have become a public health focus worldwide. Breastfeeding practices and attitudes are influenced by demographic, biophysical, social, cultural and psychological factors.[1]

More than 2.4 million infant deaths occur in India each year and two thirds of these deaths are related to inappropriate infant feeding practices.[2] In India, the National Family Health Survey -3 (NFHS Ill Survey, 2005-2006) reveals that only 46 percent children in the age group 0-6 months are exclusively breastfed.[3]

Breastfeeding provides optimal and complete nutrition for newborn babies and the health risks associated with infant formula feeding are increasingly documented.[4] During infancy, breastfeeding protects against infectious disease and in long-term, breastfeeding is associated with benefits in several areas, such as cardiovascular risk factors, intellectual capacity, and allergy.[5]

Under nutrition in children demands an urgent need for a comprehensive multi-pronged evidence based strategy to tackle the situation. Evidence based interventions, which include initiation of breastfeeding within one hour of birth, exclusive breastfeeding for the first six months of life and introduction of appropriate and adequate complementary food after 6 months of age, are available to prevent under nutrition in children and to improve child survival. Breastfeeding also provides health benefits to women, in terms of less bleeding at the time of birth, less cancers and less fractures in later ages.[6]

It is known that breastfeeding rates vary by demographic and socioeconomic factors and that these factors may impact differently on initiation and duration. Studies have indicated that characteristics such as increasing maternal age, education, income and being a non smoker are associated with higher rates of breastfeeding.[7]

The first two years of life are critical stages for a child's growth and development. Any damage caused by nutritional deficiencies during this period could lead to impaired cognitive development, compromised educational achievement and low economic productivity.[8]

Various factors associated with sub-optimal breastfeeding and complementary feeding practices have been identified in various settings. These include maternal characteristics such as age, marital status, occupation, and education level; antenatal and maternity health care; health education and media exposure; socio-economic status and area of residence; and the child's characteristics including birth weight, method of delivery, birth order, and the use of pacifiers.[8]

Each year, 5.6 million infants die because they do not receive adequate nutrition.[9] The World Health Organisation (WHO) recommends exclusive breastfeeding for first six months. The estimated reduction of infant mortality by promoting exclusive breastfeeding is 13%. Non-exclusive breastfeeding rather than exclusive breastfeeding can increase the risk of dying due to diarrhea and pneumonia among 0-5-month-old-infants by more than two-fold.[10]

Despite awareness of the many advantages of breast feeding, its rates often fall short of recommended practice. In an effort towards achieving exclusive breastfeeding practices, United Nations International Children's Emergency Fund (UNICEF) and WHO launched the Baby Friendly Hospital Initiative (BFHI) in 1991 to ensure that all maternity facilities support mothers in making the best choice about feeding.[11]

Importance of exclusive breastfeeding (EBF) is well established particularly in poor environments where early introduction of other milk is of particular concern because of the risk of pathogens, contamination and over dilution of milk leading to increased risks of morbidity and undernutrition.[12]

In India, breastfeeding in rural areas appears to be shaped by the beliefs of a community, which are further influenced by social, cultural and educational factors. Successful breastfeeding depends not only on mother's education but also on support and motivation from family members and health care professionals.[13]

India, breastfeeding is almost universal. However, the rates of early initiation, exclusive breastfeeding and timing of complementary feeds are far from desirable. There have been few only studies done on the bacteriological study of infant feeding bottles. Though the breast feeding is almost universal in India, the time of initiation of breast feeding, exclusive breast feeding, and complimentary feeding have wide variability.

This study is aimed at the awareness of breast feeding and the bacteriological study of the infant feeding bottles and he hygienic practices in inevitable conditions and its clinical application in practical pediatric practice. Aims and objectives of present study were

- To isolate and identify the aerobic bacteria from the infant feeding bottles
- To study antimicrobial susceptibility pattern of these isolates.
- Clinical application of this study to the primary care givers/ providers.
- To make aware of, and discourage the use of feeding bottles by the parents and shift to better alternatives feeding practices
- To study the prevalence of various illnesses in bottle fed babies.

METHODS

A consent was taken from the parents who fed their babies with infant feeding bottle and explained about the necessity to obtain culture of the bottles and nipples, either by culture swab scraping the inner layer and also explained about the adverse effects of bottle feeding and shift over to other alternative methods inevitable conditions.

Inclusion criteria

- All the parents who came to OPD and IPD to check up their children and have carried feeding bottle with them.
- Babies who are bottle fed for one month or more
- Age group from one month to two years of age

Exclusion criteria

- Unused and Washed bottles
- Babies who are receiving bottle feeds for less than one month or temporarily for a short period when mother is away.

All the parents who carried feeding bottles with their babies, coming to Katuri Medical College OPD and admitted in ward.

Method of collection of data including sampling procedures if any

Sterile swabs were used to obtain swipe from the feeding bottles (inner side of bottles) and nipples (inner side) of the bottle fed babies visiting the OPD and admitted in the wards and sent for microbiological assay. Hands were washed with soap and sample collection was done and transported in cold chain with at most care to prevent contamination. It was inoculated on the Mac Conkey agar culture media (plates) and incubated at 37° C for 24 hours. The first subculture will be done after 24 hours of incubation, the second on the third day and a final on the seventh day. Subcultures will be done onto 5% sheep blood agar and MacConkey agar plates. The inoculated plates will be incubated aerobically in the incubator at 37° C for 24 hours, and the plates were observed for growth [according to AIIMS -NICU protocol 2008].[2] The growth will be identified by colonial characteristics, gram's stain and standard biochemical tests described in Mackie and McCartney, Practical Medical Microbiology, and Bailey and Scott's Diagnostic Microbiology.[14,15] Cultures which did not yield any growth following three subcultures were reported negative at the end of 7 days.

Antibiotic susceptibility testing

Antibiotic susceptibility testing will be done for all the isolates on Muller Hinton agar using commercially available discs (Hi media), by Kirby Bauer disc diffusion technique. commercially available discs (Hi media), by Kirby Bauer disc diffusion technique technique as per the CLSI guidelines [2012].

Table 1: List of antibiotics.

Gram negative organisms			Gram positive organisms		
antibiotics	**Conc (^g/disk)**	**abby**	**antibiotics**	**Conc (^g/disk)**	**abby**
ceftriaxone	30	CTR	penicillin	20	P
gentamicin	30	G	Chloramphenicol	10	C
amikacin	30	AK	ciprofloxacin	5	CIP
cefotaxim	30	CE	erythromycin	15	E
Clavulanic-acid	10	CL	vancomycin	30	VA
aztreonam	20	AZ	linezolid	25	L

RESULTS

All the mother who had bottle fed their babies and had carried the feeding bottle to department of paediatrics, Katuri Medical College has been consider with respect to inclusion and exclusion criteria. In the current study, 46% were male babies and 54% female babies, showing no much difference in gender.

Table 2: Age wise distribution.

Age of initiation of bottle feeding	Number	Percentage
<6 months	32	32
6months-1 year	45	45
>1 year	23	23
Total	100	100

The Table 2 shows that 32% were initiated on bottle feeding before 6months of age and 45% during 6-12 months of age and 23% after age of 1 year.

The Table 3 shows that bottle feeding is common in mothers who are outdoor working i.e 53% and 28% in indoor working, and 19% in house wife.

Table 3: Occupation of the mother.

Occupation of mother	Number	Percentage
Out door	53	53
In door	28	28
House wife	19	19
Total	100	100

Table 4: Socio-economic status of the family.

SES	Number	Percentage
Class I-III	56	56
Class IV-V	44	44
Total	100	100

The Table 4 showed that bottle feeding is 56% in class 1/11/III according to modified kuppuswamy classification and 44% in class IV/V.

The study showed that mothers problem contributed to 74% and problems related to baby is 26%.

The Table 5 shows that most common presenting complaint was Gastrointestinal contributing to 43% and respiratory illness 39% and lastly by others by 18%.

Table 5: Presenting illness.

Presenting illness	Number	Percentage
Respiratory illness	39	39
GIT	43	43
Others (malnutrition, follow-up, immunization)	18	18

The study showed 41 % mixed feeding practice (breast milk and top milk) and 59% only top feeding (where only breast feeding was done).

The study showed formula milk (powdered) is used as a baby supplement in 27% and natural top milk (cows, buffalo, goat) contributed to 73%.

Table 6: Technique of cleaning feeding bottles.

Technique of cleaning bottle	Number	Percentage
Adequate	41	41
Inadequate	59	59
Total	100	100

The Table 6 showed that adequate cleaning practices were followed by only 41 % of mothers and 59% of mothers had poor cleaning practices.

Table 7: Weight of the babies.

Percentile	Number	Percentage
< 3rd percentile	13	13
3rd to 50th percentile	38	38
50th to 95th percentile	42	42
>95th percentile	07	07

The Table 7 shows that, 13% were <3rd percentile and 38% were <50th percentile and 42% were 50th to 95th percentile and about 7% were >95th percentile.

Table 8: Correlation between occupation and initiation of bottle feeding.

Age of initiation, of bottle feeding	Outdoor occupation	Indoor occupation	House wife	Total
<6 months	24	08	00	32
6 months-1 years	26	17	02	45
>1 year	03	03	17	23
Total	53	28	19	100

The Table 8 showed that in outdoor working mother the prevalence of bottle feeding in <6 months is 45% (24) in and 49% (26) in 6months to 12 months age group and 5.4% in >1 year of age group. In indoor working mother it is 28.5% (8) from <6months of age, 60.7% (17) in 6months to 12 months of age and 10% (3) in >1year of age group. In housewife mother it is 0% (0) in <6months of age, 10.5 (2) in 6months to 12 on this of age and 89% (17) in >1 year of age group.

Table 9: Correlation between presenting illness and age of presentation.

Age	Respiratory illness	GIT illness	Other illness	Total
<6months	15	16	01	32
6 months to 1year	19	18	08	45
>1 year	05	09	09	23
Total	39	43	18	100

The Table 9 shows that the most common complaint in bottle fed babies in first 6months of age is GIT (diarrhea, vomiting) composing to 51.2% (16) followed by respiratory illness (URTI, LRTl) 46.2% (15) and other complaints 3.8%(1) The above table showed that in age group of 6months to 12 months, respiratory illness is 48% (18) followed by GIT 41.8% (18) and others 10.2% The above study showed that prevalence of illness was less in age group of >1years.

Table 10: Comparison between socioeconomic status and adequacy of cleaning feeding bottles.

SES	Technique of cleaning bottle		Total
	adequate	In adequate	
Class I-III	34	22	56
Class IV-V	07	37	44
Total	41	56	100

The Table 10 shows that about 60. 7% of the high socioeconomic (class 1/11/111) followed the adequate cleaning practices of feeding bottle, about 39.4% (22) had poor cleaning habits. In low class (class IV/V) about 84% (37) had poor cleaning practices on infant feeding bottle, only 15% (7) followed adequate cleaning practices.

Table 11: Top v/s mixed feeding and the presenting illnesses.

Pattern of feeding	respiratory	GIT (diarrhea)	others	Total
TOP	22	26	11	59
Mixed	17	17	07	41
Total	39	43	18	100

The Table 11 showed that the incidence of illness was more in exclusively top fed babies composing of 60. 7% compared to mixed fed babies 39.3%.

The study showed that 53% of the samples are culture positive and 47% are yielded no growth.

The Table 12 shows that the most common isolate found was E-coli 45% (24) followed by Klebsiella 24% (12), Staphylococcus 16% (9), CONS 9% (5), Enterobacterecciea 3% (2) and Pseudomonas 0.8% (1).

Table 12: Isolation of the organism.

Organism present	Frequency	Percentage
E. coli	24	45
Klebsiella	12	22
Staphylococcus aureus	09	16
CONS	05	09
Enterobacter	02	03
Pseudomonas	01	01

Tables 13 and 14 shows that *E-coli* is 100% sensitive to aztreonam followed by70.8% sensitive to ceftriaxone and amikacin then followed by cefotaxim, clauvtanic acid and gentamicin.

Kliebsella is 100% sensitive to aztreonam and 81 % sensitive to ceftrixone and amikacin then followed by cefotaxim, clauvlanic acid and gentamcin. Staph is 100% sensitive to vancomycrn and linezolid, 77. 7% sensitive to Chloremphenicol and ciprofloxacin then followed by erythromycin and penicillin.

CONS is 100% sensitive to vancomycin and linezolid, 80% sensitive to erythromycin then followed by ciprofloxacin, chloramphenicol and penicillin.

Table 13: Sensitivity of each organism (Gram negative organism).

	Ceftriaxone	Gentamycin	Amikacin	Chloramphenicol	Clavulanic acid	Aztreonan
Klebsiella	09	04	09	05	07	11
Enterobacter	01	00	01	01	01	02
E. coli	17	06	16	11	05	23
Pseudomonas	01	00	00	00	00	01

Table 14: sensitivity of each organism (Gram positive organism).

	Penicillin	Chloramphenicol	ciprofloxacin	Ethambutol	vancomycin	Linezolid
S. Aureus	03	07	06	05	09	09
CONS	01	02	02	04	05	05

Enterobacter is 100% sensitive to aztreonam and 50% sensitive to Ceftrixone and amikacin, cefotaxim, clauvlanic followed by gentamcin.

Pseudomonas is 100% sensitive to aztreonam and ceftriaxone and resistant to Other commonly used antibiotics

The study showed that 52% (52) of the sample showed fungal isolation positive on microscopy and of this 73% (38) were clinically detectable.

DISCUSSION

W Infection is still a major cause of morbidity and mortality in the newborn period. it has seen that bottle feeding has been a culprit in hampering the nutritional and immune status of the infant and increased incidence of infection and contributes to under five morbidity and mortality and also malnutrition.

In the present study, attempt has been made to study the incidence of bottle feeding among infants but found to be of no much difference in respect to gender. This study showed that age of initiation of bottle feeding was found to 3% in <6 months of age group and 45% in 6 months to 1 year of age group, and 23% in >1year of age group.

This study also showed that initiation of bottle feeding below 6 months was found more in outdoor working mothers (53%) than compared to indoor working mothers (28%) and house wife mothers (19%). This study findings were similar to the study done by S B Feinet al and the other study conducted by Sima Roy and Tiwaari et al showed expecting to work full-time decreased the probability of breast-feeding (OR = 0.47, p <0.01).[16,17] Working full-time at 3 months postpartum decreased breast-feeding duration by an average of 8.6 weeks (P <0.001) relative to not working, but part-time work of 4 or fewer hours per day did not affect duration, and part-time Work of more than 4 hours per day decreased duration less than full-time work. The most common presenting illness were diarrhea (43%) followed by respiratory illness (39%) this study correlates with the study done by Kathryn G Dewey et al which showed that incidence of diarrheal illness among breast fed infants was half that of formula fed infants; the percentage with any otitis media was 19% lower and with prolonged episodes (>10 days) was 80% lower in Breast fed compared with Formula fed infants.[18] These findings are also coinciding with the study done by Tuscon and Dundee where the infants suffering from RTI was 33.6 % and diarrhea was 48.2% in limited exclusively breastfed group (<3 months) compared to 29.8% and 22.5% in prolonged exclusively breastfed group (>3 months).[19]

This study showed that the bottle feeding has contribution to the malnutrition (under nutrition and over nutrition) i.e. about 13% were <3rd Percentile, and 38% were <50th percentile and about 7% were >95th percentile (over nutrition). This study correlates with study done by Khokhar A et al. which showed top feeding a contributing factor for malnutrition in under five nutrition.

This study showed that incidence of diarrhea was more in babies who were bottle fed before 1 year of age i.e. 51.2% and 48% incidence of respiratory illness was common in babies who were bottle fed after 6months of age. This study was similar to findings of Tucson and Dundee where the number of infants suffering from RTI was 33.6% and diarrhea was 48.2% in limited exclusively breastfed group (<3 months) compared to 29.8% and 22.5% in prolonged exclusively breastfed group (>3 months).[19] Other study done by Nitin Joseph et al showed that the mean number of episodes of morbidities was least among infants who were EBF for 6 months (2.16±0.95 episodes per infant per year) as compared to infants who were either EBF for less than (4.1±1. 7 4 episodes per infant per year) or more than 6 months (4.03±1-62 episodes per infant per year).[20]

These findings also correlate with the study done by Brown KH et al which showed, Infants for whom breast-feeding was discontinued during the first 6 months had 27.6% diarrheal prevalence. During the second 6 months of life, discontinuation of breast-feeding was also associated with an increased risk of diarrheal incidence and prevalence.[21]

Most of the mother were aware of alternative feeding methods of infants like spoon, katori and palada feeding but because of time constraint and convenience they preferred bottle feeding, this correlates with the study done Mayuri M, et al were study was done on alternative feeding practices followed by mothers showed the prevalence was feeder bottle 58% bowel and spoon 32% and10% tumbler.[22]

This study showed that unhygienic practices are 84% prevalence in low economic status, which contributed to the contamination of the infant feeding bottles (p value 0.001). This study correlates with the cohort study done by Kathryn G. Dewey et al, which showed that parental socioeconomic status has influence on bottle feeding and hygienic practices which intern play role in infant morbidity. This study showed that most of the educated and well to do family had awareness of cleaning infant feeding bottle.

This study showed that low socioeconomic family preferred the top milk like cow, goat or buffalo milk with inappropriate dilution and some mother used formula milk with over dilution, and thus insufficient nutrition of the baby and thus malnutrition Which is further worsened with recurrent infection which shows that 13% are <3rd percentile, 38% are 3rd-50th percentile, 42 are 50th-95th percentile and 7% are >95th percentile. This study correlates with the study done by Mayuri M, et al which showed over dilution of top milk compromises nutritional profile of the baby.[22]

This study showed that in unhygienic practices contributed to contamination of the infant feeding bottles (p value =0.0023) and thus culture positivity was more in these bottles, contributing to the illness in these bottle fed babies. This study correlates with the study done by Nitin Joseph et al Which showed that the incidence of morbidity disorders among infants was found to decrease with increase in literacy (P = 0.231) and socio-economic status (P = 0.305) of their mothers and also study done by Banik ND et al · The incidence of morbidities in infants was found to decrease with increasing educational and socio-economic status of their mothers which was similar to the findings of study done in Delhi.[23]

This study showed that even after applying adequate cleaning practices, chances of contamination of infant feeding bottles can still persist since the organism were isolated from the bottles of the babies whose mother practiced an adequate cleaning procedure, this study correlates with study done by Redmond EC et al showed extrinsic contamination from inappropriate handling or ineffective disinfection.[24]

The incidence of illness is 60.7% in only top fed babies compared to babies who are both breast fed and bottle fed 39.3%, showing the more incidence of illness in only top fed babies. This study correlates with the study done by Arifeen S et al which tells that Exclusive Breastfeeding Reduces Acute Respiratory Infection and Diarrhea Deaths Among Infants in Dhaka Slums.[25]

The reason for bottle feeding when asked showed that it was mainly attributed to mother's problems by 74% of which most common was false perception of the mother that her milk is not sufficient for her baby, this finding correlate with the study conducted by Kulkarni et al.[26]

Oral mucosa was examined for oral thrush in all the babies who are bottle fed, 52% were shown positive (scraping was taken and studied for fungal hypae, suggestive of Candida infection) and only 38 (72%) among those were clinically detected of the total sample 53% of the samples are culture positive and 47% are yielded no growth.

Most common of gram negative isolates is *E. coli* i. e. 45 % (24) of the culture Positive isolates which correlates with study done by Erika Bergstrom.[27]

E-coli is 100% sensitive to Aztreonam followed by 70.8% sensitive to ceftriaxone and amikacin then followed by cefotaxime, clauvlanic acid and Gentamicin. Of the gram-positive organism, staph aureus was the most common isolate contrirbuting to 9% which correlate

with the study done by Redmond EC, Staphylococcus aureus was detected from 4% of bottles component.[28]

Kliebsella is 24% (12) of the culture positive isolates, Kliebsella is 100% sensitive to aztreonam and 81 % sensitive to ceftriaxone and amikacin then followed by cefotaxim, clauvlanic acid and gentamicin

Staphallococus16% (9) of the culture positive isolates, Staph is 100% sensitive to vancomycin and linezolid, 77.7% sensitive to chloremphenicol and ciprofloxacin then followed by erythromycin and penicillin

CONS 9% (5) of the culture positive isolates. CONS is 100% sensitive to vancomycin and linezolid, 80% sensitive to erythromycin then followed by ciprofloxacin, chloremphenical and penicillin

Enterobactererecciea 3% (2) of the culture positive isolates, Enterobacter is 100% sensitive to aztreonam and 50% sensitive to ceftriaxone and amikacin, Cefotaxim, clauvlanic followed by gentamcin.

The enterobacter is isolated from the bottles in which formula feed is used which accounts for 7% of the formula feed used. This isolation of Enterobacter species correlates with many studies done across the world by Iversen C et al , Himelright I et al, Van Acker et al ,these child were suffering from acute diarrhea correlating with bacteraemia, necrotising enterocolitis (NEC) and infant meningitis asepsis in infancy Muytjens, H.L et al.[29-32]

Pseudomonas 0.8% (1) of the culture positive isolates, Pseudomonas is 100% sensitive to aztreonam and ceftriaxone and resistant to other commonly used antibiotics.

This baby from whose pseudomonas was isolated had directly come to NICU with severe sepsis, abdominal dissension, severe wasting with septic shock. This baby was top fed from the day one of life as mother had expired because of obstetrics complication. This shows that there are multiple factors which contributing to the usage of bottle for infant feeding practices.

CONCLUSION

Because of lack of awareness of importance of breast feeding and advances in infant feeding practices, have been responsible of weaning of breast milk feeding and shift over to bottle feeding (top milk).

The incidence of bottle feeding was found more common in working women (53%) compared to housewife (19%)

Literate mother was resistant to wean off bottle feeding when compared to illiterate mother. Mother with household work can be convinced more easily than the working mother for the avoidance of bottle feeding.

Illiteracy and Low SES of the family contributed to the improper dilution of top milk thus compromising the nutritional requirement and causing malnutrition.

Most common reason for bottle feeding was contributed to mother's problem of which most mother complains of inadequate mother's milk to satisfy the babies hunger (false perception). Mainly the reason is improper counseling of mother about breast feeding.

Knowledge of cleaning feeding bottles was good among high class (60.7%) and some of middle class family but was poor in low class family (15%) and this contributed to the contamination of the feeding bottle

Those babies who were initiated with bottle feeding before the age of 6 mo, had frequent illness. Diarrhea (43%) to be most common in first year of like followed by respiratory illness (39%).

Oral thrush was found in most of the babies who were bottle fed, 52% were shown positive only 38 (72%) among those were clinically detected.

The organism isolated from bottles were mainly gram negative organism of which most common was is *E-coli* 45% (24) followed by Klebsiella 24% (12), Staphallococus 16% (9), CONS 9% (5), Enterobactererecciea 3% (2) and Pseudomonas 0.8% (1).

Almost all organisms were sensitive to the rarely used or higher antibiotics and almost all organisms showed resistance of varying degree in commonly used antibiotics.

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