Efficacy and Safety of Prophylactic Antifungal Agents in Preventing Invasive Fungal Infection and Mortality among Infants Weighing Less than 1500 Grams: A Meta-analysis

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ABSTRACT

Background. Preterm infants with very low birth weight are at increased risk of invasive fungal infections. Preventive strategies are needed to improve their clinical course and survival.

Objectives. To assess the efficacy and safety of antifungal agents as prophylaxis in controlling invasive fungal infection and mortality in very low birth weight (VLBW) and extremely low birth weight (ELBW) infants in neonatal intensive care units.

Methods. We searched MEDLINE (PubMed), Cochrane databases, Google Scholar, Trip database, Herdin, and ClinicalTrials.gov without language restriction and publications from January 1988 to May 2021. We included randomized controlled trials or controlled clinical trials that compared the effect of prophylactic oral or systemic antifungal agents versus placebo in preterm infants < 37 weeks age of gestation and with birth weight lower than 1500 grams. We conducted a meta-analysis using RevMan 5.4.1 and certainty of evidence rating using GRADEpro software.

Results. A total of 14 studies (including 3,001 preterm infants with VLBW) were included. We found that prophylactic use of nystatin significantly reduced the incidence of invasive fungal infections (IFI) (pooled RR 0.16; 95% CI 0.11, 0.23; 4 RCTs, N = 1295; *P* < 0.00001; moderate certainty evidence) in preterm infants compared to placebo but had no significant effect on the mortality (RR 0.87; 95% CI 0.62, 1.23; 4 RCTs, N = 1295; *P* = 0.43; low certainty evidence). Similarly, fluconazole decreased the incidence of IFI (RR 0.38; 95% CI 0.28, 0.53; *P* = 0.02) and showed statistically significant reduction in mortality (RR 0.78; 95% CI 0.61, 0.99; RCTs, N = 1484; P = 0.04; high certainty evidence). The comparison of the two antifungals showed a trend favoring fluconazole, however the difference was not statistically significant in decreasing IFI (RR 1.60; 95% CI 0.68, 3.77; *P* = 0.28) and mortality (RR 1.62; 95% CI 0.76, 3.45; *P* = 0.21).

Conclusion. Administration of antifungal prophylaxis proves to be beneficial and can probably decrease invasive fungal infection and mortality. The evidence showed that Fluconazole is superior as antifungal prophylaxis compared to placebo while there is no significant difference between fluconazole and nystatin in decreasing fungal infection and mortality among preterm neonates.

Keywords: nystatin, fluconazole, antifungal, prophylaxis, preterm, very low birth weight, meta-analysis

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INTRODUCTION

It is estimated that 15 million babies are born prematurely every year. Worldwide, prematurity is one of the leading causes of under 5 mortalities. Premature or preterm birth is when a baby is born too early before 37 weeks of pregnancy have been completed. The more premature the baby is the higher risk of death or complications.[1]

More than 60% of preterm births occur in Africa and South Asia. Around 12% of babies in lower-income countries are born too early compared with 9% in higher-income countries.[2] Globally, the mortality rate for preterm infants has improved over the last decades.[3] The Philippines in 2017 still identified prematurity and its complications as the leading cause of neonatal deaths at 31%. Infants born prematurely also remain vulnerable to many complications such as respiratory distress syndrome, bronchopulmonary dysplasia, injury to the intestine, necrotizing enterocolitis, compromised immune system, hearing and vision problems, and neurologic insult. Prematurity is the most important risk factor for nosocomial infection.[3-6] Preterm babies are likewise more susceptible to developing an invasive fungal infection as compared with full-term babies. Gestational age, male gender are also risk factors for the development of invasive fungal infection while vaginal delivery and use of antibiotics during the first week of life further increase the incidence among the more premature infants.[7] Studies also showed that colonization of the skin and gastrointestinal tract, and use of indwelling catheters are predictors of systemic fungal infections.[8] *C. albicans* and *C. parapsilosis* are the most common species isolated in episodes of invasive disease in neonates.[9]

Neonatal systemic fungal infection or invasive fungal infection is defined as fungal infection of a normally sterile body site such as blood, urine, CSF while colonization pertains to the isolation of fungi on skin or mucosal surface without evidence of invasion.[10]

Invasive fungal infections are a major cause of morbidity and mortality in neonates and both immunocompromised and immunocompetent children.[11] The rate of predisposition to invasive fungal infection correlates with the degree of the underdeveloped immune system, skin, respiratory and gastrointestinal tract.[12] Several factors, including the use of indwelling devices, broad-spectrum antibiotics, total parenteral nutrition, corticosteroids, gastrointestinal surgery, and/or history of fungal colonization, contribute to the risk.[13]

Invasive fungal infection in preterm infants typically occurs around the third week of life presenting with signs and symptoms of generalized late-onset sepsis (respiratory distress, feeding intolerance, lethargy, and hypotension), predominantly, as a result of *Candida spp*. infection.[14-16]

Local studies in the Philippines are very limited. In a study conducted in a single tertiary hospital by Sta Maria K, et al. in 2019, an overall prevalence rate of invasive candidiasis was reported at 10.24% (ELBW 15.36%, VLBW 8.11%). *Candida albicans* was isolated in 37.80% of cases while non-albicans candida (NAC) in 36.58% of neonates.[17]

Invasive fungal infection is associated with high morbidity and mortality of premature infants. The involvement of the central nervous system is a unique characteristic of invasive candidiasis among infants. The incidence of *Candida* meningitis among infants with candidemia varies from 5–25%.[18-19] Other CNS presentations such as parenchymal abscesses and vasculitis are also observed in infants with invasive candidiasis.[11] However, cerebrospinal fluid (CSF) cultures are often negative, CSF parameters (e.g., white blood cell count) are often normal, and imaging is unreliable.[8,20-21]

It is recognized that preventive strategies for invasive candidiasis are very much needed. General strategies include hand hygiene, cohorting, provision of individual room for families and newborns; reduction of risk factors for colonization and infection for invasive candidiasis, such as limitation of use of H2-receptor blockers, corticosteroids, and broad-spectrum antibiotics, mainly on the use of carbapenems and third-generation cephalosporins; minimizing the use of invasive devices including minimal manipulation of central venous catheters, as well as early introduction of mother´s milk.[22]

Several studies support the use of prophylactic antifungal agents to significantly decrease colonization and the development of invasive disease and its complication. Fluconazole is a suitable drug for prophylaxis owing to its characteristics of long half-life and high CSF penetration. It is metabolized by the liver; 80% is excreted unchanged in the urine. These characteristics allow for long dosing intervals, excellent tissue penetration, and easy elimination.[23]

The Infectious Diseases Society of America (IDSA) recommends intravenous or oral fluconazole prophylaxis given as 3–6 mg/kg twice weekly for 6 weeks, in neonates with birth weights < 1000 g among nurseries with high rates (> 10%) of invasive fungal infection, Oral nystatin, 100,000 units 3 times daily for 6 weeks, is an alternative to fluconazole in neonates with birth weights < 1500 g when Fluconazole is not available or there is suspected azole resistance.[24] Various Spanish scientific societies (Spanish Society of Infectious Diseases and Clinical Microbiology – SEIMC and Spanish Society of Pediatric Infectious Diseases – SEIP) likewise advocate the use of fluconazole prophylaxis at 3 mg/kg/day in newborns with birth weight < 1,500 g, continuing it for all the period at risk. The European Society of Clinical Microbiology Infectious Diseases (ESC-MID) strongly recommends it in the Neonatal Intensive Care Unit (NICU) with a prevalence higher than 5% of invasive candidiasis in babies < 1,000 g at birth at a dose of 3–6 mg/kg twice weekly intravenously or orally.[25] The Latino American Working Group of invasive fungal infections also recommends fluconazole prophylaxis 3 mg/kg twice a week, in newborns weighing < 1,000 g for six weeks in NICU with a prevalence of invasive candidiasis > 5%.[26]

Although currently available evidence and foreign recommendations show fluconazole as an effective prophylaxis treatment against invasive fungal infections in preterm neonates in the NICUs, the timing, duration, and dosing remain controversial. At present routine giving of fluconazole or any other antifungal drug as prophylaxis in neonates is not routine in all local institutions.[27-31] The Philippines has yet to come up with a guideline that will recommend the need and use of antifungal prophylaxis.

Nystatin is considered to have a comparable prophylactic effect. It has the advantage of not being systemically absorbed, allowing sufficient contact with colonizing fungal agents in the GI tract. It is also non-toxic, easy to use, and less expensive as compared with any other antifungal agents including fluconazole.[31-33]

This systematic review and meta-analysis aimed to assess the efficacy and safety of antifungal agents as prophylaxis in controlling invasive fungal infection and mortality in very low birth weight (VLBW) and extremely low birth weight (ELBW) infants in neonatal intensive care units.

MATERIALS AND METHODS

Criteria for considering studies for this review

Type of studies

We included clinical trials, randomized controlled trials, meta-analyses, and systematic reviews. There was no language restriction applied in the search.

Type of participants

Preterm infants < 37 weeks age of gestation and with birth weight lower than 1500 g

Type of intervention

Antifungal prophylaxis with nystatin and fluconazole versus no antifungal prophylaxis or use of placebo

Type of outcome measures

Primary outcomes

1. Confirmed invasive fungal infection
2. Mortality
3. Safety
4. Dose of systemic (intravenous) fluconazole prophylaxis
5. Interval of systemic (intravenous) fluconazole prophylaxis

Secondary outcomes

1. Fungal colonization
2. Mean NICU stay
3. Bronchopulmonary dysplasia (oxygen supplementation at 36 weeks postmenstrual age)
4. Necrotizing enterocolitis
5. Bacterial sepsis

Search methods for identification of studies

Electronic searches

We searched MEDLINE (PubMed), Cochrane databases, Google Scholar, Tripdatabase, Herdin, and ClinicalTrials.gov without language restriction and publications from January 1988-May 2021. Keywords used were "prematurity," "very low birth weight infants," "VLBW," "extremely low birth weight," "ELBW," "candida," "invasive candidiasis," "fungal", "invasive fungal infection", "antifungal prophylaxis," "fluconazole prophylaxis," and "nystatin prophylaxis" (Supplementary Table 1).

The search was performed in duplicate by two researchers.

Searching other resources

We also did forward citation tracking, cross-references, and hand searching of bibliographies.

Data Collection and analysis

Selection of studies

Two reviewers (SVA and KBB) independently screened titles and abstracts of all studies identified by the above search strategy. We assessed the full-text reports of any potentially eligible records and excluded those studies that did not meet all of the inclusion criteria. We discussed any disagreements until we achieved consensus.

Data extraction and management

A data collection form was made to extract relevant information from each included study. Two review authors extracted the data separately. Disagreements were discussed until a consensus was reached.

Assessment of risk of bias in included studies

We used the criteria and standard methods of the Cochrane Handbook to assess the methodological quality of any included trials. We evaluated and reported the following issues in the 'Risk of bias's tables.

Statistical Analysis / Assessment of heterogeneity

Treatment effects of individual trials and heterogeneity between trial results were assessed by inspecting the forest plots. We calculated the I^2 statistic for each meta-analysis to quantify inconsistency across studies and describe the percentage of variability in effect estimates that may be due to heterogeneity rather than to sampling error. If we detected substantial heterogeneity (I^2 more than 50%), we explored the possible causes (e.g., differences in study design, participants, interventions, or completeness of outcome assessments).

Data synthesis

We used the fixed-effect model in Review Manager 5.4.1 for meta-analysis. For data that yielded inestimable results due to zero or missing events, imputation was done

to complete the data set and analyze as if it were complete and determine the trend.

Subgroup analysis

A subgroup analysis based on the dose, frequency, and birth weight was done with findings of significant heterogeneity in the studies included.

Assessment of overall certainty of evidence

The Grading of Recommendations, Assessment, Development, and Evaluations (GRADE) approach was used to determine the certainty of evidence.

RESULTS

Description of studies

Results of the literature search

Initially, 93 relevant documents were identified of which 83 were identified through database searches and 10 identified through hand searching of bibliographies. Seventy-two were included after the duplicates were removed while 54 were screened, 24 of which were excluded for not meeting the inclusion criteria. Thirty full-text articles were assessed for eligibility. After reading the full text, we excluded 15 non-RCT articles and one study that used a different antifungal. A total of 14 studies were included in the qualitative and quantitative synthesis (Supplementary Figure 1).

Description of studies

We included 14 eligible trials: Aydemir 2011; Benjamin 2014; Jannatdoust 2015; Kaufman 2001; Kaufman 2005; Kirpal 2016; Kicklighter 2001; Manzoni 2007; Mersal 2013; Ozturk 2006; Parikh 2007; Rundjan 2020; Sims 1988; Violaris 2010 (Supplementary Table 2).

Included studies

1. Oral Prophylaxis versus Placebo or No Drug (Comparison 1)

Four trials compared oral/topical non-absorbed antifungal prophylaxis with placebo or no drug:

- Sims 1988 quasi-randomly allocated 67 infants of birth weight less than 1250 grams to receive either oral nystatin or no treatment until one week after endotracheal extubation (average five weeks).
- Ozturk 2006 randomly allocated 938 VLBW infants to receive either prophylactic oral nystatin (100,000 IU three times daily) or no treatment. Infants in the control group who had oral fungal colonization detected at trial entry or on surveillance cultures were treated with nystatin (100,000 IU three times daily).
- Aydemir 2011a randomly allocated 185 VLBW infants to receive either oral nystatin 100,000 IU three times daily or "equal volumes of intravenous or oral normal saline" placebo every third day until the 30th day after birth (or 45th day in ELBW infants).
- Rundjan 2020 randomly allocated 95 VLBW preterm infants to receive either oral nystatin 100,000 IU/mL 1mL (0.5 mL coated in the oral cavity and another 0.5 mL was given through orogastric tube) three times daily or 1 mL sterile water three times daily for six weeks.

The primary outcomes of all studies were fungal colonization and invasive fungal infection. All provided data on in-hospital mortality but none assessed any post-discharge outcomes.

2. Systemic (intravenous) Fluconazole Prophylaxis versus Placebo or No Drug (Comparison 2)

Eight trials compared systemic fluconazole prophylaxis with placebo or no drug. The trials were subdivided according to dose: 6 mg/kg dose versus 3 mg/kg dose.

- Kicklighter 2001 randomized 103 VLBW infants to receive either fluconazole 6 mg/kg every three days or a placebo for the first 28 days of life. Outcome data on invasive fungal infection and mortality were reported.
- Manzoni 2007 randomized 322 VLBW infants to receive either fluconazole 6 mg/kg every two days or 3 mg/kg every 2 days or placebo.
- Parikh 2007 randomly assigned 120 VLBW to receive either 6 mg/kg every three days or a placebo. Outcome data on invasive fungal infection and mortality were reported.
- Benjamin 2014 randomized 361 VLBW to receive either 6 mg/kg 2x a week and placebo for 6 weeks. Outcome data on invasive fungal infection and mortality were reported.
- Kaufman 2001 randomly allocated 100 less than 1,000 gm to receive either fluconazole 3 mg/kg every 1–3 days or placebo. Outcome data on invasive fungal infection and mortality were reported.
- Aydemir 2011c randomly allocated 184 VLBW infants to receive either intravenous fluconazole 3 mg/kg every third day until 30 days after birth or "equal volumes of intravenous or oral normal saline" placebo (or 45 days after birth in ELBW infants).
- Jannatdoust 2015 randomly assigned 93 preterm neonates less than 1250 g to receive either fluconazole 3 mg/kg every third day for two weeks, every two days for two weeks, and daily for another two weeks or no treatment. Outcome data on invasive fungal infection and mortality were reported.
- Kirpal 2016 randomly assigned 75 preterm neonates less than 1500 g to receive either fluconazole 6 mg/kg every third day or a placebo. Outcome data on invasive fungal infection, mortality, and length of hospitalization were reported.

3. Oral Nystatin versus Systemic Fluconazole Prophylaxis (Comparison 3):

Three trials compared oral/topical antifungal prophylaxis with systemic antifungal prophylaxis:

- Violaris 2010 randomized 80 VLBW infants to receive either oral nystatin or fluconazole beginning between days five to seven after birth. Outcome data on invasive fungal infection and mortality were reported.
- Aydemir 2011b randomly allocated 187 VLBW infants to receive either oral nystatin 100,000 IU eight hourly or intravenous fluconazole 3 mg/kg every third day until 30 days after birth (or 45 days after birth in ELBW infants).
- Mersal 2013 randomly allocated 59 preterm infants of birth weight less than 1200 grams to receive either oral nystatin 100,000 IU eight hourly for six weeks (N = 24) or intravenous fluconazole 6 mg/kg every 72 hours at end of the first week of life, then every 48 hours from the second week to the sixth week of life (N = 35).

Safety (Comparison 4):

No study discussed the adverse effects of nystatin. Three studies discussed the elevation of liver enzymes after the use of fluconazole prophylaxis: Benjamin 2014, Kirpal 2016 and Manzoni 2007.

Dose of systemic (intravenous) fluconazole prophylaxis (Comparison 5)

Manzoni 2007 randomized 322 VLBW infants to receive either fluconazole 6 mg/kg every 2 days or 3 mg/kg every 2 days or placebo.

Interval of systemic (intravenous) fluconazole prophylaxis (Comparison 6)

Kaufman 2005 randomly assigned 81 preterm neonates less than 1,000 gm to receive either fluconazole 3 mg/kg every 72 hours for two weeks, every other day for two weeks, and daily for another two weeks or fluconazole 3 mg/kg twice a week for six weeks. Outcome data on invasive fungal infection and mortality were reported.

Excluded studies

We excluded 16 studies (Weiner 1992; Howell 2009; Bertini 2005; Healy 2005; Uko 2006; Aghai 2006; McCrossan 2007; Healy 2008; Weitkamp 2008; Aziz 2010; Rueda 2010; Martin 2011; Rolnitsky 2012; Cetinkaya 2014; Lee 2016; Dalili 2020).

Risk of bias in included studies

Quality assessments are described in the table characteristics of included studies and risk of bias (Supplementary Table 3) are displayed in Figure 1. Most of the studies included were randomized, double-blind controlled trials. In one study by Jannatdoust et al., randomization was not explained and Sims et al. used quasi-randomization; hence, there was a high risk of bias. In three studies (Mersal, Sims, and Violaris), the participants, personnel, and assessors were not blinded resulting in classification as high risk for performance and detection bias. In the study by Mersal, the two deaths that were initially excluded seemed to be in the fluconazole group but vaguely discussed; hence, was considered high risk for reporting bias.

Effects of interventions

Oral/topical Nystatin prophylaxis versus placebo or no drug (Comparison 1)

Primary outcomes

Invasive fungal infection (Outcome 1.1). Four RCTs compared oral nystatin to placebo for the prevention of invasive fungal infection. All these studies demonstrated a decline in invasive fungal infection among preterm term infants favoring the intervention of giving oral nystatin prophylaxis versus placebo, and the difference was statistically

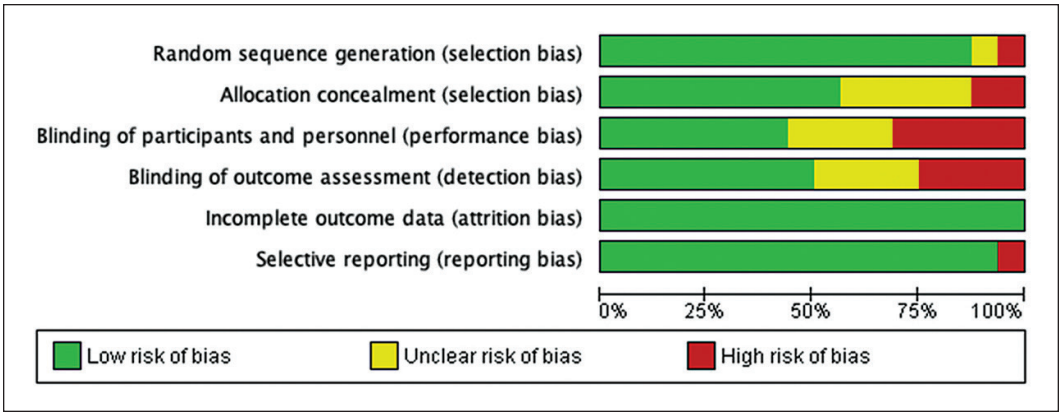


Figure 1. Risk of bias graph: Review of authors' judgments about each risk of bias item presented as percentages across all included studies.

significant (RR = 0.16; 95% CI 0.11, 0.23). There was no significant heterogeneity as the study population was similar across the four studies, infants included were very low birth weight < 1500 g, given the same amount and frequency of oral nystatin used at every 8 hours and the initiation of treatment at 72 hours of life. The study by Ozturk et al. had the biggest number of patients while Rundjan et al. had the least (Figure 2).

Mortality (Outcome 1.2): In terms of mortality as an outcome for nystatin versus placebo, all of the studies did not find a significant effect in terms of decreasing mortality. In the pooled analysis, 56 out of 649 babies randomized to the nystatin group died compared to 64 out of 646 randomized to the placebo group. Visually, there seems to be less number of babies who died under the nystatin group, however, the results showed no statistical difference in mortality between nystatin and placebo (P = 0.43) (Figure 3).

Secondary Outcomes

Fungal Colonization (Outcome 1.3). Three RCTs compared oral nystatin to placebo for the prevention of fungal colonization All studies demonstrated a decline in fungal colonization among preterm infants favoring the intervention of giving oral nystatin prophylaxis versus placebo, and the difference was statistically significant with a risk ratio of 0.16 (95% CI 0.25, 0.51). There was no significant heterogeneity among the three studies (I^2 = 40%) (Figure 4).

Mean NICU Stay (Outcome 1.4). Two trials reported length of stay in the intensive care unit (Sims 1988; Aydemir 2011a). The pooled data in 252 patients seem to favor giving nystatin prophylaxis in terms of shortening the length of NICU stay, however, none of the trials reported a statistically significant difference; RR -1.19 (95% CI -7.27, 4.89).

Bronchopulmonary dysplasia (Outcome 1.4). Aydemir 2011a did not find a statistically significant difference: RR 1.29 (95% CI 0.67 to 2.49). Outcome not reported in the other trials.

Necrotizing enterocolitis (Outcome 1.5). Aydemir 2011a did not find a statistically significant difference: RR 0.97 (95% CI 0.40 to 2.33). Outcome not reported in the other trials.

IV Fluconazole Prophylaxis versus Placebo or No Drug (Comparison 2)

Primary outcomes

Invasive fungal infection (Outcome 2.1). Eight studies compared the effect of fluconazole vs placebo on the

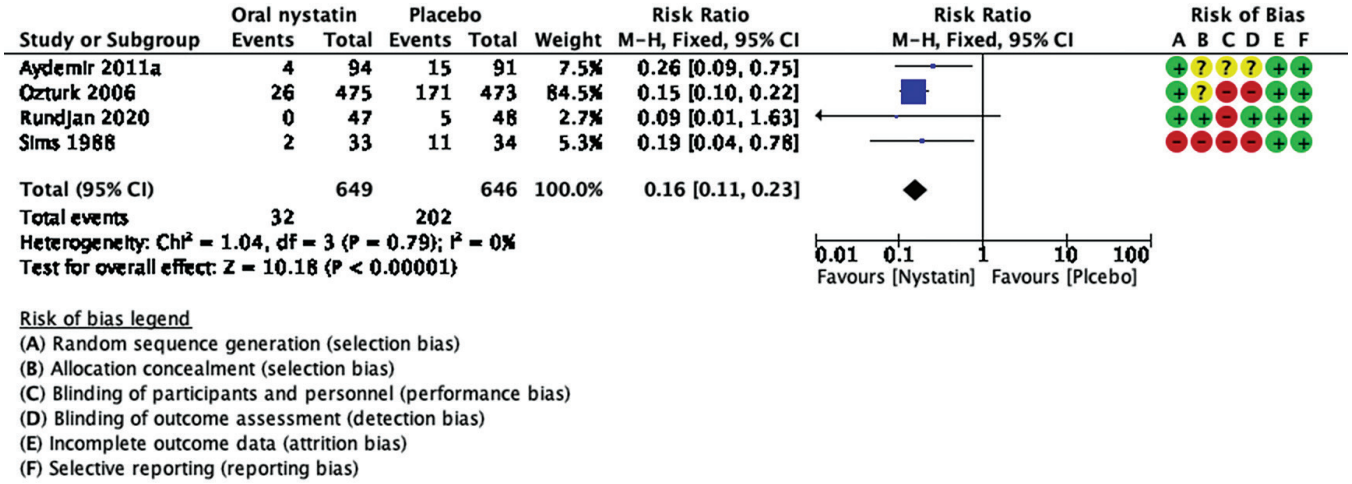


Figure 2. The effect of nystatin on the incidence of invasive fungal infection.

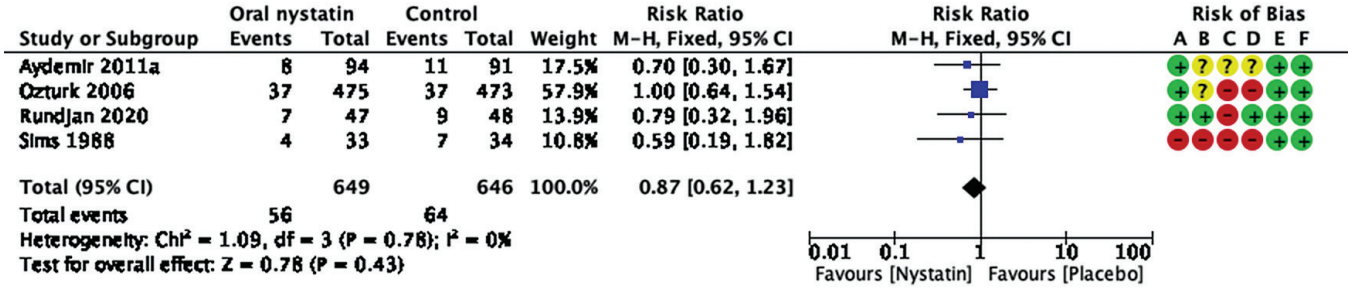


Figure 3. The effect of nystatin on mortality.

incidence of IFI. The result of the meta-analysis revealed that the occurrence of IFI in the group using fluconazole prophylactically was significantly lower than the placebo group, and the difference was statistically significant with an RR = 0.38 (95% CI 0.28, 0.53). When we analyzed according to the dose there was no note of heterogeneity in the 3 mg/kg dose but those subanalyzed in the 6 mg/kg dose showed significant heterogeneity (I^2 = 56%) (Figure 5).

When we visually inspect the plot, we can see that the values generated from the study of Parikh crossed the line of null effect which seems to favor placebo. When we did our sensitivity analysis, we found that the heterogeneity was altered with the exclusion of Parikh. Results of Parikh can be attributed to the reporting of one more neonate in the Fluconazole prophylaxis group as compared with placebo.

Mortality (Outcome 2.2). Among the eight studies included, the overall number of deaths was 96 in the Fluconazole group while there were 123 in the placebo group. The trend favors giving fluconazole as prophylaxis to decrease neonatal deaths. The overall mortality in the group with prophylactic use of fluconazole was significantly lower than in the placebo group (RR 0.78; 95% CI 0.61, 0.99) (*P* = 004). There was homogeneity across the studies (I^2 = 0%) (Figure 6).

Secondary Outcome

Fungal Colonization (Outcome 2.3). The result of the meta-analysis revealed that the occurrence of fungal colonization in the group using fluconazole prophylactically was significantly lower than the placebo group, and the difference was statistically significant (RR 0.36; 95% CI 0.25, 0.52). When we analyzed according to the dose there was no note of heterogeneity in the 3 mg/kg and 6 mg/kg dose (I^2 = 0%) (Figure 7).

Bacterial Sepsis (Outcome 2.4). The result of the meta-analysis revealed that the occurrence of bacterial sepsis in the two groups (fluconazole and placebo) seemed to be similar, with most studies within the line of no effect. Statistically, there was also no significant difference between the two groups (RR 0.97; 95% CI 0.84, 1.11). When we analyzed according to the dose there was no note

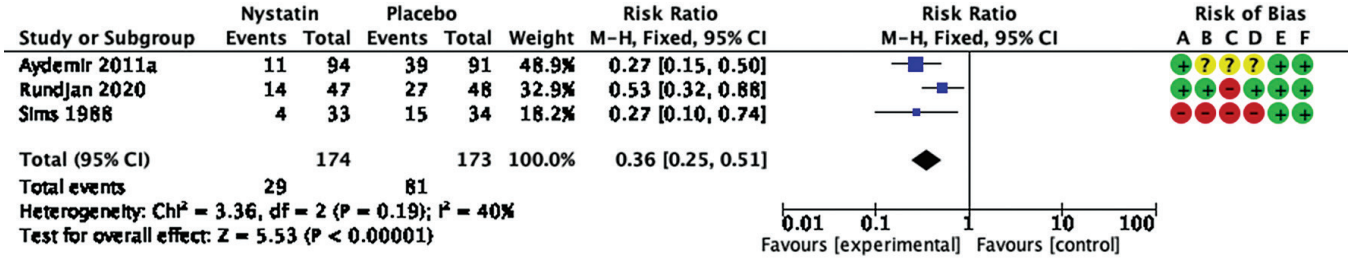


Figure 4. The effect of nystatin on fungal colonization.

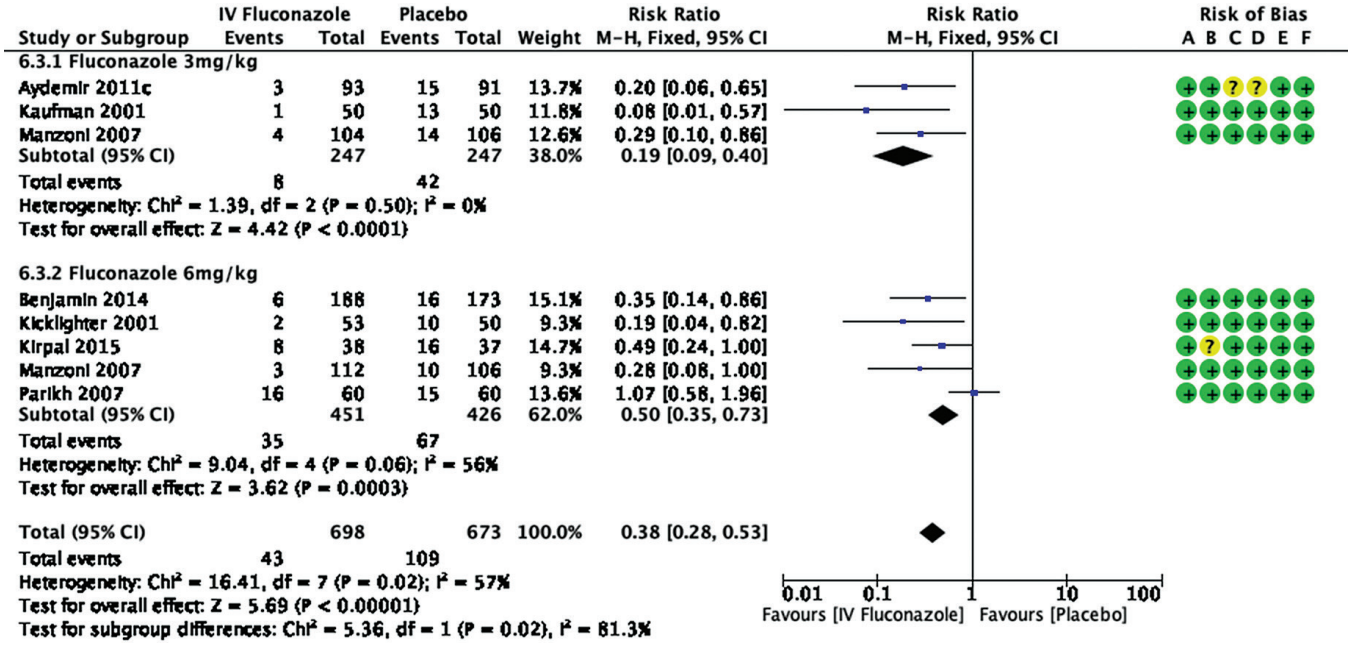


Figure 5. The effect of fluconazole vs placebo on invasive fungal infection.

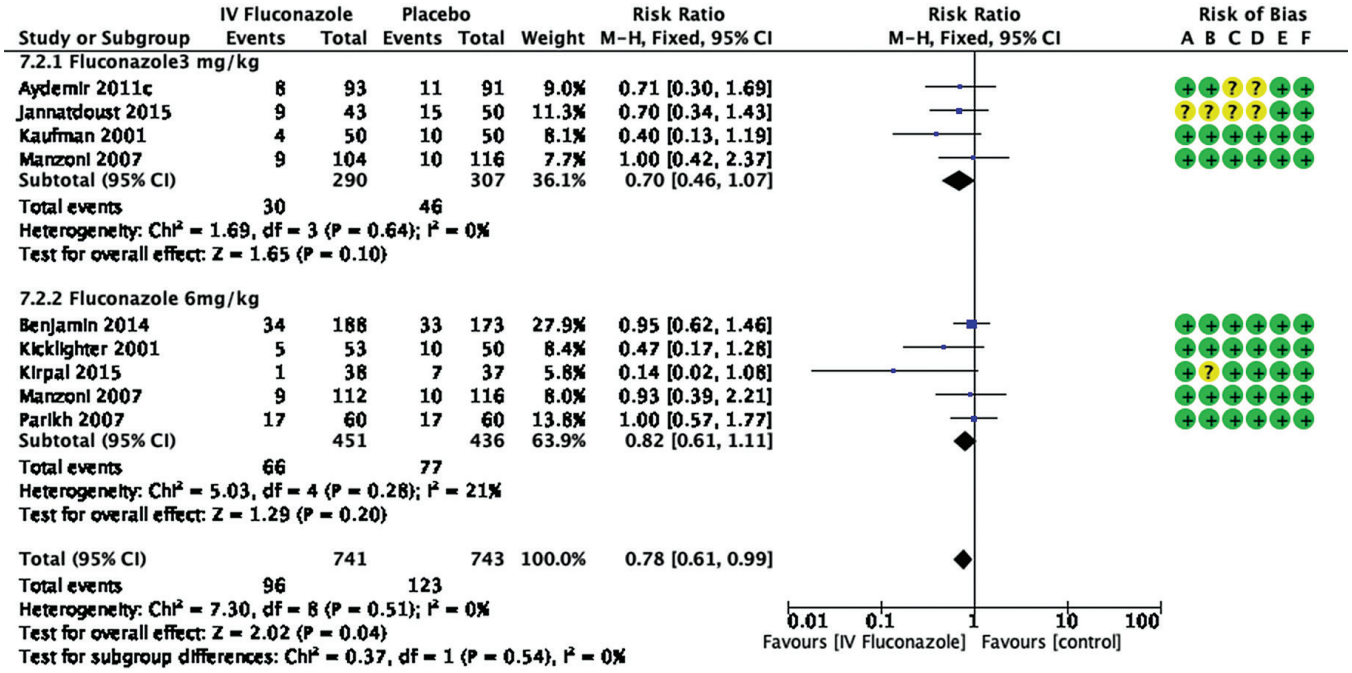


Figure 6. The effect of fluconazole vs placebo on mortality.

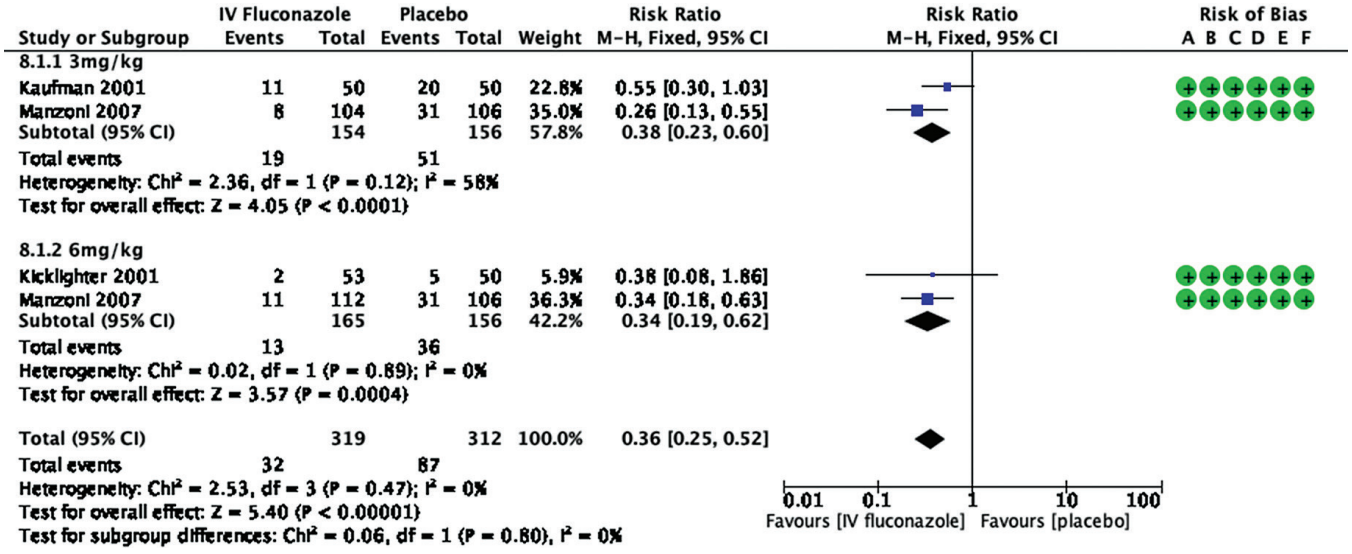


Figure 7. The effect of IV fluconazole vs placebo on fungal colonization.

of heterogeneity between the 3 mg/kg and 6 mg/kg dose (I^2 = 0%) (Figure 8).

Oral Nystatin versus IV Fluconazole Prophylaxis (Comparison 3)

Primary outcomes

Invasive fungal infection (Outcome 3.1). The trend favors fluconazole in decreasing IFI over nystatin in the three studies, however, no statistical difference was noted between the two groups. Imputation was done to complete the data set and analyze as if were complete and determined the trend due to the not estimable result in Mersal 2013. Imputation showed similar findings wherein visually, the trend favored fluconazole; however, there was no statistical difference between the two groups (P = 0.28). There was no heterogeneity among the studies (I^2 = 0%) (Figure 9).

Mortality (Outcome 3.2). More deaths were observed in the nystatin group as compared to the fluconazole group. However, there was no significant difference between the two antifungal agents (P = 0.23). There was heterogeneity

across the studies, which can be due to the sample size and the causes of death that need further investigation as to whether it is attributable to an adverse event from antifungal or be clarified on death due to prematurity complications (Figure 10).

Secondary Outcomes

Mean NICU Stay (Outcome 3.3). Aydemir 2011b did not find a statistically significant difference with regards to the mean NICU stay between the two groups: MD

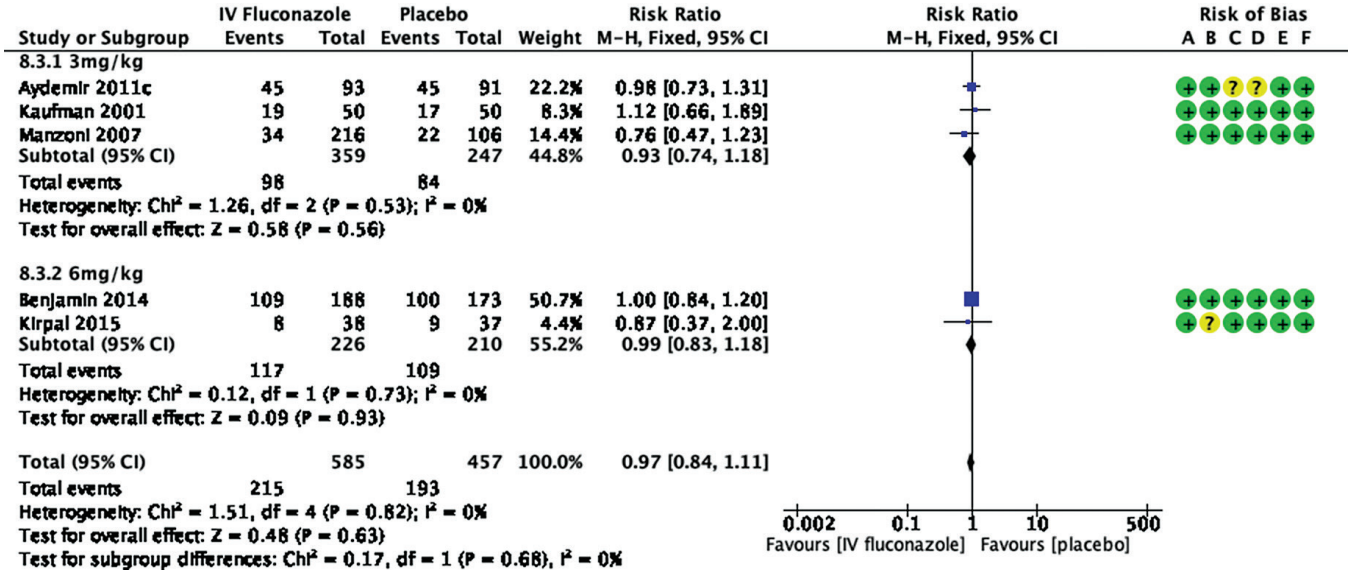


Figure 8. The effect of IV fluconazole vs placebo on bacterial sepsis.

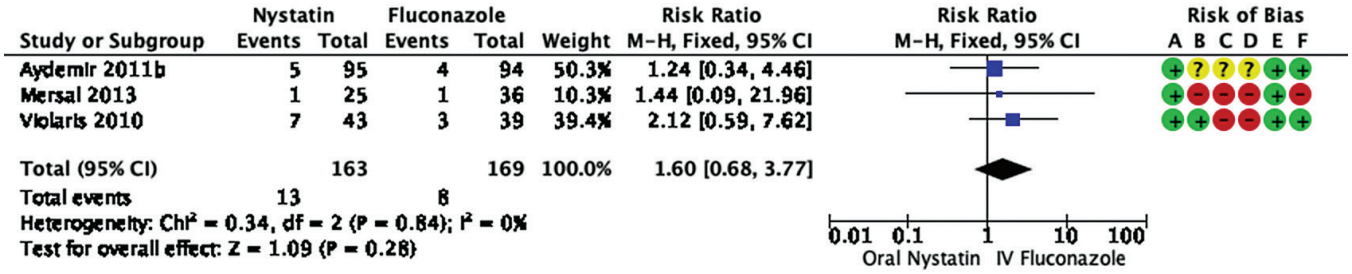
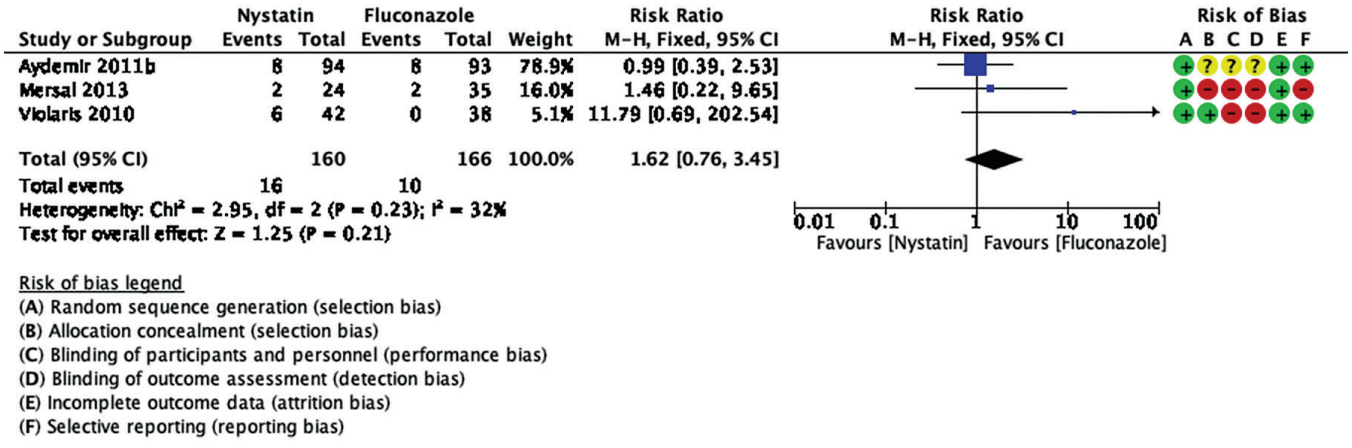


Figure 9. The effect of nystatin vs IV fluconazole on invasive fungal infection.



Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding of participants and personnel (performance bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)

Figure 10. The effect of nystatin vs IV fluconazole on mortality.

–1.00 (95% CI –7.63 to 5.63) days. This was not reported by Violaris 2010 or Mersal 2013.

Bronchopulmonary dysplasia (Outcome 3.4). Aydemir 2011b did not find a statistically significant difference: RR 1.29 (95% CI 0.67, 2.49]. This outcome was not reported by Violaris 2010 or Mersal 2013.

Necrotizing enterocolitis (Outcome 3.5). Meta-analysis of data from Violaris 2010 and Aydemir 2011b did not detect a statistically significant difference in the number of neonates developing necrotizing enterocolitis in the two groups (RR 1.22; 95% CI 0.58, 2.60; I^2 = 0%). This outcome was not reported by Mersal 2013.

Sepsis (Outcome 3.6). Meta-analysis of data from Violaris 2010 and Aydemir 2011b did not detect a statistically significant difference in the neonate's developing sepsis in those given nystatin and fluconazole (typical RR 1.05, 95% CI 0.80, 1.83). The data however showed statistically significant heterogeneity (I^2 = 83%). This outcome was not reported by Mersal 2013.

Safety (Comparison 4)

The common side effects of nystatin included mouth irritation, nausea, vomiting, diarrhea or. skin rash. These were not measured among the studies on oral nystatin. Elevated liver enzymes were reported in three studies using fluconazole prophylaxis but did not statistically differ from placebo.

In the study of Benjamin et al., there were 4/188 (2%) infants in the fluconazole group and 3/173 (2%) in the placebo group who had elevated aspartate aminotransferase (AST) or alanine aminotransferase (ALT) greater than 250 U/L; glutamyl transpeptidase (GGT) > 100 U/L in 38/188 (20%) among those infants with fluconazole prophylaxis versus 37/173 infants (21%) in the placebo; conjugated bilirubin > 5 mg/dl 15/188 (8%) fluconazole group versus 20/173 (12%) in the no antifungal group.

Kirpal et al. reported that 2 out of 38 in the fluconazole prophylaxis group and 1 out of 37 in the control group developed AST/ALT elevation of more than 3 times the normal (P > 0.05). No statistically significant adverse effects were observed.

There were no significant differences in the levels of AST, ALT, GGT and bilirubin in the study by Manzoni et al. Among those given 3 mg/kg fluconazole for four weeks, 2x elevation of AST was observed in 2/104 (1.9%) infants, 0 in placebo; 2x increased ALT in 2/104 (1.9%) and 0 in placebo; 2x elevated GGT in 5/104 (4.8%) versus 6/106 (5.7%) in placebo; bilirubin > 5 mg/dl 2/104 (1.9%) fluconazole group, 1/106 (0.9%) placebo. Infants given 6 mg/kg fluconazole 2x increased AST in 2/104 (1.8%), 0 placebo; 2x elevated ALT seen among 2/104 (1.8%), 0 in placebo; 2x GTT levels in 8/104 (7.1%), 6/106 (5.7%) in placebo; > 5 mg/dl bilirubin in 1/104 (0.9%) and 1/106 (0.9%) for those who received fluconazole and no antifungal prophylaxis respectively.

Dose, Interval and Duration of Nystatin and Fluconazole (Comparison 5)

There was no study comparing different doses of nystatin and there was only one study by Manzoni et al. that compared different doses of fluconazole (3 mg/kg and 6 mg/kg). The incidence of invasive fungal infection was 2.7% in the 6 mg group, 3.8% in the 3 mg group, and 13.2% in the placebo group (P = 0.005 for the 6 mg group and P = 0.02 for the 3 mg group vs. the placebo group). The overall mortality was similar among groups.

One study by Kaufman et al. compared fluconazole 3 mg/kg every 72 hours for two weeks, every other day for two weeks, daily for two weeks (Group A), and twice a week for six weeks (Group B). Fungal bloodstream infection/ invasive fungal infection occurred in two (5%) Group A and one (2.5%) Group B patient (risk difference, 0.02; 95% CI, 20.14, 0.10; P = 0.68).

GRADE Evidence Profile (Tables 1–3)

Oral nystatin prophylaxis versus placebo or no drug (Comparison 1)

Nystatin prophylaxis showed clear benefit and the evidence was deemed moderate due to the risk of bias. In three studies, the assessors and personnel were not blinded which can make them at risk for performance and detection bias. The certainty of evidence in the mortality outcome was further downgraded due to the wide confidence interval.

Fluconazole prophylaxis versus placebo or no drug (Comparison 2)

Fluconazole clearly showed a benefit compared to placebo with high certainty evidence. The beneficial use of fluconazole as prophylaxis is reflected in the decrease in the number of patients who developed invasive fungal infections and deaths as compared to placebo.

Oral nystatin versus systemic (intravenous) fluconazole prophylaxis (Comparison 3)

For the nystatin vs fluconazole prophylaxis, it was assessed to be of clear benefit but the certainty of the evidence was moderate, downgraded due to risk of bias. The methods of random sequence generation and allocation concealment were not stated and the assessors and personnel were not blinded in one study. Two studies did not explain how the participants, assessors, and personnel were blinded making it at risk for performance and detection bias. The nystatin group had more patients who developed invasive fungal infections than the fluconazole group.

DISCUSSION

Prevention of invasive fungal infection is critical for the survival of preterm infants, decreases the likelihood of complications, end-organ damage, and lifelong sequelae.

Table 1. GRADE evidence profile of nystatin prophylaxis vs placebo or no drug on invasive fungal infection and mortality

Certainty assessment							Summary of findings				
Participants (studies) Follow-up	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall certainty of the evidence	Study event rates (%) With Placebo	Study event rates (%) With Nystatin	Relative effect (95% CI)	Anticipated absolute effects Risk with Placebo	Anticipated absolute effects Risk difference with Nystatin
Invasive Fungal Infection											
1295 (4 RCTs)	serious[a]	not serious	not serious	not serious	none	⨁⨁⨁◯ MODERATE	202/646 (31.3%)	32/649 (4.9%)	**RR 0.16** (0.11 to 0.23)	313 per 1,000	**263 fewer per 1,000** (from 278 fewer to 241 fewer)
Mortality											
1295 (4 RCTs)	serious[a]	not serious	serious[b]	not serious	none	⨁⨁◯◯ LOW	64/646 (9.9%)	56/649 (8.6%)	**RR 0.87** (0.62 to 1.23)	99 per 1,000	**13 fewer per 1,000** (from 38 fewer to 23 more)

CI: confidence interval; RR: risk ratio

Explanations
[a] *One study was quasi-randomized hence at risk for selection bias; three studies did not have participants, assessors, and administrators hence risk for performance and detection bias*
[b] *Wide confidence interval*

Table 2. GRADE evidence profile of fluconazole prophylaxis vs placebo or no drug on invasive fungal infection and mortality

Certainty assessment							Summary of findings				
Participants (studies) Follow-up	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall certainty of the evidence	Study event rates (%) With Placebo	Study event rates (%) With Fluconazole	Relative effect (95% CI)	Anticipated absolute effects Risk with Placebo	Anticipated absolute effects Risk difference with Fluconazole
Invasive Fungal Infection											
1371 (7 RCTs)	not serious	not serious	not serious	not serious	none	⨁⨁⨁⨁ HIGH	109/673 (16.2%)	43/698 (6.2%)	**RR 0.38** (0.28 to 0.53)	162 per 1,000	**100 fewer per 1,000** (from 117 fewer to 76 fewer)
Mortality											
1484 (8 RCTs)	not serious	not serious	not serious	not serious	none	⨁⨁⨁⨁ HIGH	123/743 (16.6%)	96/741 (13.0%)	**RR 0.78** (0.61 to 0.99)	166 per 1,000	**36 fewer per 1,000** (from 65 fewer to 2 fewer)

CI: confidence interval; RR: risk ratio

Table 3. GRADE evidence profile of oral nystatin vs fluconazole prophylaxis on invasive fungal infection and mortality

Certainty assessment							Summary of findings				
Participants (studies) Follow-up	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall certainty of the evidence	Study event rates (%) With Fluconazole	Study event rates (%) With Nystatin	Relative effect (95% CI)	Anticipated absolute effects Risk with Fluconazole	Anticipated absolute effects Risk difference with Nystatin
Invasive Fungal Infection											
326 (3 RCTs)	serious[a]	not serious	not serious	not serious	none	⨁⨁⨁◯ MODERATE	5/166 (3.0%)	10/160 (6.3%)	**RR 1.89** (0.66 to 5.39)	30 per 1,000	**27 more per 1,000** (from 10 fewer to 132 more)
Mortality											
326 (3 RCTs)	serious[a]	not serious	not serious	not serious	none	⨁⨁⨁◯ MODERATE	10/166 (6.0%)	16/160 (10.0%)	**RR 1.62** (0.76 to 3.45)	60 per 1,000	**37 more per 1,000** (from 14 fewer to 148 more)

CI: confidence interval; RR: risk ratio

Explanation
[a] *The study of Volaris did not explain how randomization and allocation was done, participants, personnel, and assessors were unblinded*

Administration of antifungal prophylaxis proves to be beneficial and can decrease invasive fungal infection and mortality.

Nystatin and fluconazole are among the antifungal agents in current clinical use.[34] Nystatin, a polyene, is the earliest antifungal drug primarily by the oral route. It is active against many species of yeast and candida albicans and is widely used to treat skin and oropharyngeal candidiasis. Nystatin is not absorbed orally and has not been linked to drug-induced liver injury.[35] Fluconazole, on the other hand, is a well-tolerated triazole with good activity against *Candida spp.* except *C. krusei* and *C. glabrata*. In the prophylactic setting, fluconazole has proven efficacy for primary prevention of invasive candidiasis in high-risk patients with leukemia, bone marrow, liver transplantation and cryptococcosis, and recurrent mucosal candidiasis in Acquired Immunodeficiency Syndrome (AIDS). The use of fluconazole as prophylaxis should, however, be limited to selected high-risk patients to prevent the emergence of azole-resistant strains.[36]

The current meta-analysis of data from four randomized clinical trials (Sims 1988, Ozturk 2006, Aydemir 2011, and Rundjan 2020) suggested that oral nystatin decreases colonization as well as the risk of invasive fungal infection in VLBW infants significantly. However, none of these showed a substantial effect on mortality. In the included studies the causes of death were not fully explained. The findings, therefore, must be treated with caution. More robust studies with larger sample sizes and improved methodological quality (e.g., blinding of assessors and personnel) are needed to strengthen the recommendation on the beneficial use of nystatin among preterm infants. Based on available data, there is no established effective timing and duration in using nystatin for high-risk infants.

The most reliable and methodological evidence is in favor of the use of fluconazole in preventing colonization, invasive fungal infection, and mortality. In most of the studies regardless of the dosage, the benefit of fluconazole was seen. Only one study by Manzoni et al. was able to compare different dosages of fluconazole compared to placebo. Based on the limited available data it was difficult to determine the magnitude and effect of the different dosages (3 mg/kg vs 6 mg/kg) on fungal colonization, invasive infection, and mortality. However, the use of either 3 mg/kg or 6 mg/kg fluconazole was superior to not giving anti-fungal prophylaxis at all. Protective or preventive level achieved by using a smaller dose of fluconazole at 3 mg/kg may offer the advantage of decreasing the likelihood of azole-resistance organisms and maybe more cost-saving. However, this has yet to be seen in further studies.

In terms of the interval between doses, there was only one study by Kaufman et al. which examined the effect of the different intervals of giving fluconazole. In the study, 81 preterm neonates less than 1,000 gm were randomly assigned to receive either fluconazole 3 mg/kg every 72 hours for two weeks, every other day for two weeks, and every day for another two weeks or fluconazole 3 mg/kg twice a week for six weeks. Both schedules revealed a reduction in invasive fungal infection and mortality but favor the twice-weekly regimen for six weeks. With the given data, recommendation on the interval dosing of fluconazole at this time is difficult to establish.

Aside from invasive or systemic fungal infection and death, prematurity as earlier mentioned predisposes an infant to other serious complications. In the included studies in this meta-analysis, other outcomes measured seen were bronchopulmonary dysplasia (BPD), necrotizing enterocolitis (NEC), bacterial sepsis and mean length of stay in the NICU. Their length of stay (LOS) in hospital is influenced primarily by their gestational age (GA) at birth and medical conditions leading to longer stays.

With the onset of bronchopulmonary dysplasia or NEC (necrotizing enterocolitis) among preterm infants and association with antifungal prophylaxis, the study of Aydemir et al. showed that there were no significant differences between those who received nystatin prophylaxis versus no drug for both outcomes measured. Two trials (Sims 1988, Aydemir 2011) demonstrated shorter mean NICU stay among infants given nystatin prophylaxis versus placebo. Results are inconclusive on the effect of antifungal prophylaxis on the length of NICU stay of preterm infants. These observations can be attributed to small sample sizes in the studies.

Among infants who developed bacterial sepsis given nystatin or fluconazole or placebo, the incidence of bacterial sepsis seemed to be lower with the use of fluconazole but data is not sufficient to conclude.

Nystatin and fluconazole are relatively safe and well-tolerated. Literature reports common adverse effects of nystatin are gastrointestinal upset and rashes which are not often observed. Fluconazole on the other hand has been associated with elevated liver enzymes and jaundice. Hepatoxicity, however, was not related to the amount or duration of treatment.[37] Rarely, angioedema, hypokalemia, leucopenia, neutropenia, thrombocytopenia was seen. In the studies on antifungal prophylaxis, there were no reported clinically significant adverse effects with the use of nystatin or fluconazole but findings were limited to only three studies (Manzoni 2007, Kirpal 2016 and Benjamin 2014) which included safety as an outcome measure. The authors did not find any statistical safety concerns associated with the use of fluconazole. No study mentioned any safety signals on nystatin.

The goal of caring for sick preterm infants should be to promote normal growth and development and minimize morbidity and mortality especially while they are admitted to the NICU. Advances in medicine and research result in better neonatal outcomes. Fungal infection continues to be a problem and often aggravates the frail condition of preterm infants. Fluconazole has been proven to be beneficial in

preventing and controlling the systemic fungal infection and even death. Local guidelines should consider including this antifungal agent as part of the armamentarium in the management of preterm. Identification of preterm infants who are at high risk of infection and dying from fungal infection should be prioritized.

Limitations of the study

This meta-analysis included studies involving only two types of prophylactic anti-fungal agents, nystatin and fluconazole as these are the commonly used drugs. Available studies which are randomized controlled trials have also limited these drugs. The authors found two studies that looked into Amphotericin B and Miconazole as prophylaxis but were excluded because these were observational studies.

Recommendations

Future targets for research should include optimum dosing schedule, frequency, and duration of antifungal prophylaxis. A cost-benefit analysis should be done to evaluate the value of giving antifungal prophylaxis among preterm neonates and also consider the type of antifungal to be used. It is also noteworthy to evaluate the risk factors associated with progression to invasive fungal infection and death. Further investigation is suggested on the tolerability and safety profile and the possibility of the emergence of resistance to the prophylactic use of nystatin and fluconazole.

CONCLUSION

Fungal infection among neonates causes a significant burden in the NICU. Administration of antifungal prophylaxis proves to be beneficial and can decrease invasive fungal infection and mortality. The evidence showed that fluconazole is superior to placebo as antifungal prophylaxis in decreasing fungal infection and mortality among preterm neonates. Although nystatin demonstrated a potential to decrease fungal colonization and invasive infection, the lack of robust data limits the recommendation of its use as an alternative to fluconazole as prophylaxis. More studies on nystatin with bigger sample sizes and improvement in methodological quality of studies are warranted.

Supplementary Materials

The supplementary figure and tables are available upon request from the authors.

Statement of Authorship

Both authors contributed in the conceptualization of work, acquisition and analysis of data, drafting and revising and approved the final version submitted.

Author Disclosure

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